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(19)(CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Modified Epimorphin

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ABSTRACT

Disclosed herein are a modified epimorphin obtained by adding a hydrophilic peptide composed of 5 to 99 amino acids to at least one terminus of a polypeptide containing the functional domain of epimorphin, and a modified epimorphin composed of a polypeptide having a structure that a hydrophobic domain adjacent to the C-terminus of the whole-length epimorphin consisting of a coiled coil domain (1) on the N-terminal side, a functional domain (2) at the center, a coiled coil domain (3) on the C-terminal side and the hydrophobic domain (4) adjacent to the C-terminus has been deleted from the whole-length epimorphin, and at least part of amino acids have been deleted from the terminal side of at least one of the coiled coil domains (1) and (3) as well. The invention also discloses a variant modified epimorphin obtained by making partial substitution, deletion and/or insertion of amino acids in the amino acid sequence of the modified epimorphin, wherein the variant maintains the function of the original sequence. The invention further discloses DNAs encoding the modified epimorphin and variant thereof, recombinant vectors containing the DNAs, transformants obtained by introducing the recombinant vectors, and a production method of the modified epimorphin and the variants thereof making use of the transformant.

TITLE OF THE INVENTION:

MODIFIED EPIMORPHIN

FIELD OF THE INVENTION

The present invention relates to a modified epimorphin obtained by modifying epimorphin, which is a polypeptide existing in mesenchymal cells and controlling the morphogenesis of epithelial tissue, or a fragment thereof, and more particularly to a modified epimorphin obtained by modifying epimorphin, which is useful in elucidating the attack mechanism of diseases caused by the morphogenetic abnormality of epithelial tissue and developing diagnosis and medical treatment for these disease and remedies for wounds, or a fragment thereof while keeping its activity, and variants (variant modified epimorphin polypeptides) obtained by making partial substitution, deletion and/or insertion of amino acids in the amino acid sequence of the modified epimorphin.

The present invention also relates to DNAs encoding such a modified epimorphin and variants thereof, recombinant vectors containing the DNAs, transformants obtained by introducing the recombinant vectors, and a production method of the modified epimorphin and the variants thereof making use of the transformant.

In the present invention, the term "modified epimorphin" means a fragment or polypeptide of epimorphin, which is obtained by modifying the epimorphin or a

fragment thereof.

BACKGROUND OF THE INVENTION

Since the normal organization and morphogenesis of epithelial tissue are under the some control of a factor originating in mesenchymal cells, and diseases attributable to the morphogenetic abnormality of the epithelial tissue may be often caused by the mesenchymal cells present around the tissue, studies on the mechanism of the mesenchymal cells which support the morphogenesis of the epithelial tissue have been made for long. Although studies on the isolation, purification and structural analysis of a molecule, which controls the morphogenesis of the epithelial tissue, have been extensively made throughout the world, however, its substance has been scarcely known under the circumstances because the object of study is a substance which expresses under restrictions of time and space in a complicated system, and so it is difficult to produce a simplified experimental system.

In order to realize the elucidation of diseases caused by the morphogenetic abnormality of the epithelial tissue and the attack mechanism thereof, and the development of medical treatments for these disease, it was an indispensable premise to isolate and purify such a molecule, which controls the morphogenesis of the epithelial tissue, and make the structure thereof clear.

Therefore, an important problem in the art was to early achieve the elucidation of the structure of such a molecule, and the like.

In such circumstances, the present inventors recently succeeded in isolation and identification of a molecule (the present inventors termed it "epimorphin") which controls the morphogenesis of the epithelial tissue (Japanese Patent Application Laid-Open No. 25295/1994). Epimorphin is a physiologically active substance comprising, as a core protein, a protein composed of 277 to 289 amino acids and is principally biosynthetically produced by mesenchymal cells.

The present inventors succeeded in determining the amino acid sequences of human and mouse epimorphin molecules. The splicing of their genes has revealed that at least three types exist in respective epimorphin molecules. The human epimorphin molecules include three types, human epimorphin represented by SEQ ID NO. 1, human epimorphin (isoform A) represented by SEQ ID NO. 2 and human epimorphin (isoform B) represented by SEQ ID NO. 3, all shown in SEQUENCE TABLE, which will be described subsequently. The mouse epimorphin molecules include three types, mouse epimorphin represented by SEQ ID NO. 4, mouse epimorphin (isoform A) represented by SEQ ID NO. 5 and mouse epimorphin (isoform B) represented by SEQ ID NO. 6, all shown in the SEQUENCE TABLE. The human epimorphin molecules and the mouse epimorphin molecules

have homology of about 90% with each other at the amino acid level. Therefore, they are well conserved even between different animal species.

However, these epimorphin molecules involved a problem that since they firmly bind to a cell membrane at a domain (hereinafter referred to as "the C-terminal hydrophobic domain) adjacent to the C-terminus thereof, which is extremely high in hydrophobic nature, while taking a complex high-order structure in the living body so as to perform their functions, they are extremely difficult to prepare while keeping the activity of epimorphin at a high level. In particular, the epimorphin and the isoform A markedly show such a tendency. When a cell membrane-binding domain exists, it is difficult to secrete epimorphin produced by cultured animal cells into a medium so as to isolate and purify it. The present inventors proposed the preparation of a soluble modified epimorphin by a process of removing the C-terminal hydrophobic domain, and the like (Japanese Patent Application Laid-Open No. 25295/1994). However, these processes have been yet insufficient in the compatibility of the maintenance of high activity with the solubility, and there has hence been a demand for development of a more improved process.

If a modified epimorphin easy to prepare and purify can be obtained while keeping the physiological activity of epimorphin, it is useful in elucidating the attack

mechanism of diseases caused by the morphogenetic abnormality of the epithelial tissue and developing medical treatments for these disease.

OBJECT AND SUMMARY OF THE INVENTION

It is an object of the present invention to provide a modified epimorphin which highly keeps the activity of epimorphin and is easy to prepare and purify.

The present inventors have analyzed the constitutive amino acids of epimorphin by a computer. As a result, it has been found that epimorphin is roughly divided into four structurally-characteristic domains as illustrated in FIG. 1. More specifically, an epimorphin polypeptide can be divided into a coiled coil domain (1), a functional domain (2), another coiled coil domain (3) and a C-terminal hydrophobic domain (4) from the N-terminal side thereof. The two coiled coil domains can be further divided into some subdomains (for example, heptad repeats and other domains).

The present inventors have also found that when a hydrophilic peptide composed of 5 to 99 amino acids is added to the N-terminus and/or C-terminus of a polypeptide containing the functional domain (2) of epimorphin, a modified epimorphin which highly keeps the activity of epimorphin and can be easily purified can be provided.

The present inventors have further found that when at least part of the coiled coil domains (1) and (3) are

deleted from an epimorphin polypeptide from which the C-terminal hydrophobic domain has been deleted, its physiological activity can be enhanced, and consequently, the balance between the physiological activity and the solubility can be adjusted at one's desire. According to this method, a modified epimorphin useful for the development of diagnosis and medical treatment for diseases caused by the morphogenetic abnormality of epithelial tissue, or the development of novel remedies for wounds and the like can be obtained without adversely affecting the high-order structure and activity of epimorphin.

The present invention has been led to completion on the basis of these findings.

According to the present invention, there is thus provided a modified epimorphin obtained by adding a hydrophilic peptide composed of 5 to 99 amino acids to at least one terminus of a polypeptide containing the functional domain of epimorphin.

According to the present invention, there is also provided a modified epimorphin composed of a polypeptide having a structure that a hydrophobic domain adjacent to the C-terminus of the whole-length epimorphin consisting of a coiled coil domain (1) on the N-terminal side, a functional domain (2) at the center, a coiled coil domain (3) on the C-terminal side and the hydrophobic domain (4) adjacent to the C-terminus has been deleted from the

whole-length epimorphin, and at least part of amino acids have been deleted from the terminal side of at least one of the coiled coil domains (1) and (3) as well.

This modified epimorphin may be a variant (variant modified epimorphin) obtained by making partial substitution, deletion and/or insertion of amino acids in the amino acid sequence thereof.

According to the present invention, there are further provided DNAs encoding the modified epimorphin and variant thereof, recombinant vectors containing the DNAs, transformants obtained by introducing the recombinant vectors, and a production method of the modified epimorphin and the variants thereof making use of the transformant.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates the structural feature of epimorphin and the design of its fragments.

FIG. 2 is an electrophoretogram obtained by preparing the fragments shown in FIG. 1 using Escherichia coli and analyzing them by SDS-PAGE.

FIG. 3 illustrates a result that functional epimorphin fragments has been detected from the fragments shown in FIG. 2 using an antibody binding to the functional site of epimorphin.

FIG. 4 illustrates the design drawing of epimorphin fragments used in Example 4.

FIG. 5 illustrates the structural feature of an epimorphin fragment (1).

FIG. 6 illustrating a relationship between activity and solubility of epimorphin fragments.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Features of the present invention will hereinafter be described in detail.

Epimorphin is a mesenchymal cell membrane molecule essential for the morphogenesis of epithelia in the fetal period and is also considered to participate in the construction of vital tissues. Epimorphin is a membrane protein identified as an antigen molecule recognized by a monoclonal antibody MC-1, which inhibits the normal morphogenesis of epithelia in various fetal tissues [Cell, Vol. 69, p. 471-481 (1992)].

Epimorphin is a protein (molecular weight: about 33 kDa) composed of about 280 amino acids. As human epimorphin molecules, there have been known epimorphin represented by SEQ ID NO. 1, epimorphin isoform A represented by SEQ ID NO. 2 and epimorphin isoform B represented by SEQ ID NO. 3, all shown in the SEQUENCE TABLE. As mouse epimorphin molecules, there have been known epimorphin represented by SEQ ID NO. 4, epimorphin isoform A represented by SEQ ID NO. 5 and epimorphin isoform B represented by SEQ ID NO. 6, all shown in the SEQUENCE TABLE. The mouse epimorphin is useful in, for

example, elucidating the attack mechanism of diseases caused by the morphogenetic abnormality of the epithelial tissue making use of model animals. The human epimorphin is useful in, for example, diagnosing and treating such diseases.

Epimorphin exists in mesenchymal cells around the epithelial tissue and has a function of controlling the morphogenesis of the epithelial tissue, and the like. Such an epimorphin firmly binds to a cell membrane at a C-terminal hydrophobic domain in its molecule while taking a complex stereostructure so as to perform its functions. The predicted product of cDNA of epimorphin is a molecule having a molecular weight of about 33 kDa. It has been already revealed that this molecule forms a plurality of SDS-resistant complexes in the living body, and that of these, an extracellular substance having a molecular weight of about 150 kDa is recognized by the monoclonal antibody MC-1.

According to an analysis by a computer, epimorphin can be roughly divided into 4 domains in structure as illustrated in FIG. 1. The domains other than the C-terminal hydrophobic domain (transmembrane domain) are composed of fragments [coiled coil domains (1) and (3)] on the N-terminal and C-terminal sides, which are easy to take the so-called coiled coil structure with hydrophobic amino acids forming regular lines (heptad repeats), and a central fragment (2) between them, which has substantially

the same size as the former fragments. The coiled coil domains (1) and (3) can be each divided into 4 subfragments including 2 heptad repeats. For reference, the detailed structure of the fragment (1), which is a coiled coil domain, is illustrated in FIG. 5.

According to the results of the study carried out by the present inventors, the central fragment (2) has been found to be a functional domain. The fact that the central fragment (2) of epimorphin is the functional domain can be judged from its reactivity with the monoclonal antibody MC-1 and cellular adhesiveness. As illustrated in FIG. 1, the fragments (1), (2), (3), (12), (13), (23), (123) and (123C: the whole-length epimorphin) were separately prepared with Escherichia coli in accordance with the method known per se in the art to determine their reactivity with the monoclonal antibody MC-1 which binds to the functional site of epimorphin. In particular, the fragments (2) and (23) showed strong reactivity (see Referential Example 1). Since the monoclonal antibody inhibits the activity of the extracellular epimorphin in the living body, this central fragment (2) is considered to be closely related to the activity of epimorphin. The cellular adhesiveness can be determined by, for example, coating dishes (not subjected to a treatment for cell culture) with solutions of the respective fragments in 8 M urea, thoroughly washing the dishes with 8 M urea and PBS (phosphate-buffered saline)

to thinly and evenly spread the fragments on the dishes, and then seeding epithelial cells on the dishes to determine their responsibility to the fragments. As a result, it has been found that the epithelial cells rapidly adhere only to the dish coated with the central fragment (2). In addition, it has also been found that the adhering phenomenon of the central fragment (2) to the epithelial cells is inhibited by the addition of the monoclonal antibody MC-1. Namely, it has been revealed that the domain of the central fragment (2) directly act on the cells.

Accordingly, it is apparent that the central fragment (2) is the functional domain of epimorphin. Epimorphin and fragments (polypeptides) containing this functional domain are expected to apply to various uses making good use of the epimorphin activity thereof. However, some of epimorphin and the functional domain-containing fragments may be low-soluble or insoluble in physiological solutions. When a polypeptide containing the functional domain of epimorphin is hardly soluble or insoluble in physiological solutions, for example, it is difficult to secrete such a polypeptide produced by cultured animal cells into a medium so as to isolate and purify it, and its handling and development to various applications also become difficult.

According to the present invention, a hydrophilic peptide composed of 5 to 99 amino acids is added to at

least one terminus of a polypeptide containing the functional domain of epimorphin, whereby a modified epimorphin which highly keeps the activity of epimorphin and is soluble in physiological solutions can be obtained.

According to the results of the analysis of the amino acid sequence of epimorphin by a computer, in the case of the human epimorphin, the functional domain of epimorphin has been found to be a domain containing an amino acid sequence ranging from the 99th amino acid (phenylalanine) to the 189th amino acid (glutamine) from the N-terminus in common to the epimorphin, the epimorphin isoform A and the epimorphin isoform B. Later, the results of a further investigation as to the human epimorphin have revealed that even a domain containing an amino acid sequence ranging from the 104th amino acid to the 187th amino acid from the N-terminus shows the epimorphin activity. Therefore, the functional domain in the human epimorphin is a domain containing an amino acid sequence ranging from the 99th amino acid to the 189th amino acid, preferably from the 104th amino acid to the 187th amino acid from the N-terminus of the whole-length epimorphin.

In the case of the mouse epimorphin, the functional domain of epimorphin has been found to be a domain containing an amino acid sequence ranging from the 100th amino acid (cysteine) to the 190th amino acid (glutamine) from the N-terminus in common to the epimorphin, the

epimorphin isoform A and the epimorphin isoform B. Later, the results of a further investigation as to the mouse epimorphin have revealed that even a domain containing an amino acid sequence ranging from the 105th amino acid to the 188th amino acid from the N-terminus shows the epimorphin activity. Therefore, the functional domain in the mouse epimorphin is a domain containing an amino acid sequence ranging from the 100th amino acid to the 190th amino acid, preferably from the 105th amino acid to the 188th amino acid from the N-terminus of the whole-length epimorphin.

<Modified epimorphin obtained by adding a hydrophilic peptide to at least one terminus of a polypeptide containing the functional domain of epimorphin>

No particular limitation is imposed on the polypeptide containing the functional domain of epimorphin so far as it contains the central domain of the epimorphin molecule, which is a functional domain. Such a polypeptide may have any length within limits of epimorphin. For example, a polypeptide composed of the whole-length epimorphin may be included. In general, it is however preferable from the viewpoints of solubility and activity that the C-terminal hydrophobic domain (a domain composed of 23-24 amino acids) be deleted from the whole-length epimorphin. It is also preferable that at least part of amino acids (amino acid residues or peptide) in the coiled coil domain (1) on the N-terminal side,

epimorphin isoform A and the epimorphin isoform B. Later, the results of a further investigation as to the mouse epimorphin have revealed that even a domain containing an amino acid sequence ranging from the 105th amino acid to the 188th amino acid from the N-terminus shows the epimorphin activity. Therefore, the functional domain in the mouse epimorphin is a domain containing an amino acid sequence ranging from the 100th amino acid to the 190th amino acid, preferably from the 105th amino acid to the 188th amino acid from the N-terminus of the whole-length epimorphin.

<Modified epimorphin obtained by adding a hydrophilic peptide to at least one terminus of a polypeptide containing the functional domain of epimorphin>

No particular limitation is imposed on the polypeptide containing the functional domain of epimorphin so far as it contains the central domain of the epimorphin molecule, which is a functional domain. Such a polypeptide may have any length within limits of epimorphin. For example, a polypeptide composed of the whole-length epimorphin may be included. In general, it is however preferable from the viewpoints of solubility and activity that the C-terminal hydrophobic domain (a domain composed of 23-24 amino acids) be deleted from the whole-length epimorphin. It is also preferable that at least part of amino acids (amino acid residues or peptide) in the coiled coil domain (1) on the N-terminal side,

which the domain forms the main cause to mask the activity of the functional domain, be deleted from the whole-length epimorphin according to the kind of the hydrophilic polypeptide to be added. In order to obtain a modified epimorphin high in solubility and easy to handle, it is desirable that at least part of amino acids in the coiled coil domain (3) on the C-terminal side be deleted from the whole-length epimorphin. At least parts of amino acids in the coiled coil domains (1) and (3) on both N-terminal and C-terminal sides may be deleted.

In particular, when amino acids in the coiled coil domain (1) on the N-terminal side of epimorphin are deleted from the N-terminal side, the epimorphin activity of the resulting fragment shows a tendency to enhance. In order to obtain a modified epimorphin higher in activity, therefore, it is preferable that at least part of amino acids in the coiled coil domain (1) on the N-terminal side of epimorphin be deleted from the whole-length epimorphin from the N-terminal side. On the other hand, when amino acids in the coiled coil domain (1) are deleted from the N-terminal side, the solubility of the resulting fragment in physiological solutions shows a tendency to reduce. However, the addition of the hydrophilic peptide to at least one terminus of such a fragment makes it possible to enhance the solubility of the fragment while keeping high activity.

In the case of the human epimorphin, the coiled coil

domain (1) is a domain ranging from the N-terminus to the 103th amino acid in the whole-length epimorphin. Fragments obtained by deleting 1 to 28 amino acids in such a domain from the N-terminal side can keep good solubility and have enhanced epimorphin activity. Fragments obtained by deleting 29 to 77 amino acids, further, 29 to 103 amino acids in such a domain from the N-terminal side can exhibit higher activity. A domain ranging from the 29th amino acid to the 103th amino acid from the N-terminus contains heptad repeats and is particularly easy to erect a coiled coil structure. Thirty to 98 amino acids from the N-terminal side may be deleted. In any case, it is preferable to delete the C-terminal hydrophobic domain.

In the case of the mouse epimorphin, the coiled coil domain (1) is a domain ranging from the N-terminus to the 104th amino acid in the whole-length epimorphin. Fragments obtained by deleting 1 to 29 amino acids in such a domain from the N-terminal side can keep good solubility and have enhanced epimorphin activity. Fragments obtained by deleting 30 to 78 amino acids, further, 30 to 104 amino acids in such a domain from the N-terminal side can exhibit higher activity. A domain ranging from the 30th amino acid to the 104th amino acid from the N-terminus contains heptad repeats and is particularly easy to erect a coiled coil structure. Thirty to 99 amino acids from the N-terminal side may be deleted. In any case, it is preferable to delete the C-terminal hydrophobic domain.

The coiled coil domain (3) is a domain obtained by removing the coiled coil domain (1), the functional domain (2) and the C-terminal hydrophobic domain from the whole-length epimorphin and varies according to the species and isoforms of the epimorphin. When the coiled coil domain (3) in the epimorphin other than the isoforms is deleted, it is generally preferable that 25 to 99 amino acids be deleted from the whole-length epimorphin from the C-terminal side.

The hydrophilic peptide to be added to the polypeptide containing the functional domain of epimorphin is a hydrophilic peptide of such a size that the solubility of the functional domain of epimorphin is improved and the exhibition of its function is not blocked. Specifically, it is a hydrophilic peptide composed of 5-99 amino acids.

Amino acids can be roughly divided into hydrophilic amino acids and hydrophobic amino acids. The hydrophilic amino acids include glycine (Gly), threonine (Thr), trypthphan (Trp), serine (Ser), tyrosine (Tyr), proline (Pro), histidine (His), glutamic acid (Glu), glutamine (Gln), aspartic acid (Asp), asparagine (Asn), lysine (Lys) and arginine (Arg). The hydrophobic amino acids include isoleucine (Ile), valine (Val), Leucine (Leu), phenylalanine (Phe), cysteine (Cys), methionine (Met) and alanine (Ala).

The hydrophilic peptide useful in the practice of

the present invention is a peptide containing bound hydrophilic amino acids in a proportion of, generally, at least 50%, preferably, at least 60%, more preferably, at least 70% based on the number of amino acids. In order to embed the resulting modified epimorphin in vivo, a hydrophilic peptide low in antigenecity may preferably be selected from the viewpoint of safety. In the case where the resulting modified epimorphin is investigated in vitro, a hydrophilic peptide easy to detect may preferably be selected. In any case, it is desirable that the hydrophilic peptide be designed so as to contain such an amino acid sequence that the purification of the resulting modified epimorphin can be performed with ease.

When a hydrophilic peptide containing at least one amino acid sequence selected from the group consisting of, for example, the following amino acid sequences (a) to (h) is used where the in vitro investigation is performed, the resulting modified epimorphin can be detected with good reproducibility because an antibody having high sensitivity to such a hydrophilic peptide is easily available. Two or more of these amino acid sequences may be contained in a hydrophilic peptide.

(a) Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala (i.e., YPYDVPDYA),

(b) Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu-Asn (i.e., EQKLISEEDLN),

(c) Glu-Tyr-Lys-Glu-Glu-Glu-Glu-Lys (i.e.,

EYKEEEEK),

(d) Tyr-Thr-Asp-Ile-Glu-Met-Asn-Arg-Leu-Gly-Lys (i.e., YTDIEMNRLGK),

(e) Arg-Ile-Gln-Arg-Gly-Pro-Gly-Arg-Ala-Phe-Val-Thr-Ile-Gly-Lys (i.e., RIQRGPGRAFVTIGK),

(f) Ala-Ser-Met-Thr-Gly-Gly-Gln-Gln-Met-Gly-Arg (i.e., ASMTGGQQMGR),

(g) Gln-Pro-Glu-Leu-Ala-Pro-Glu-Asp-Pro-Glu-Asp (i.e., QPELAPEDPED), and

(h) Gly-Ala-Pro-Val-Pro-Tyr-Asp-Pro-Leu-Glu-Pro-Arg (i.e., GAPVPYDPLEPR).

The size and kind of the hydrophilic peptide to be added to the N-terminus and/or the C-terminus of the polypeptide containing the functional domain of epimorphin may be freely chosen for combination as necessary for the intended application of the resulting modified epimorphin. Various kinds of modified epimorphin obtained by separately adding different hydrophilic peptides may be suitably mixed before use. If for example, at least five histidine (His) residues are consecutively contained in the hydrophilic peptide, the modified epimorphin prepared from this can be purified by one step through a column in which nickel has been fixed.

The hydrophilic peptide is added to the N-terminus, C-terminus or both termini of the polypeptide containing the functional domain of epimorphin. Whether the hydrophilic peptide is added to one terminus or to both

termini can be suitably determined in view of the solubility of the polypeptide containing the functional domain of epimorphin in physiological solutions, the degree of epimorphin activity of the polypeptide, the kind of the hydrophilic peptide, and the like.

If the polypeptide containing the functional domain of epimorphin is composed of the whole-length epimorphin, the hydrophilic peptide may preferably be added to both termini thereof. If the polypeptide containing the functional domain of epimorphin is a fragment obtained by deleting the C-terminal hydrophobic domain, the hydrophilic peptide may preferably be added to either the N-terminal or both termini.

If the polypeptide containing the functional domain of epimorphin is a fragment obtained by deleting at least part of amino acids in the coiled coil domain (1) from the whole-length epimorphin from the N-terminal side, the hydrophilic peptide may preferably be added to either the N-terminal or both termini in order to avoid reduction in the solubility. Even if the polypeptide containing the functional domain of epimorphin is a fragment obtained by deleting at least part of amino acids in the coiled coil domain (1) from the whole-length epimorphin from the N-terminal side and moreover deleting the C-terminal hydrophobic domain, the hydrophilic peptide may also be preferably added to either the N-terminal or both termini.

If the polypeptide containing the functional domain

of epimorphin is a fragment obtained by deleting the C-terminal hydrophobic domain and at least part of amino acids in the coiled coil domain (3) on the C-terminal side from the whole-length epimorphin, the hydrophilic peptide may preferably be added to either the C-terminal or both termini.

As a method of adding the hydrophilic peptide to one or both termini of the polypeptide containing the functional domain of epimorphin, there may be used various techniques such as biochemical techniques and gene engineering techniques. As an example of a method making use of a biochemical technique, may be mentioned a process in which an epimorphin molecule itself is chemically or physically cleaved to obtain a fragment containing the functional domain, the fragment is purified, a hydrophilic peptide is then bound to the N-terminus and/or C-terminus of the fragment making use of a chemical reaction.

As an example of a method making use of a gene engineering technique, may be mentioned a process in which a part of a gene encoding epimorphin and a gene encoding a hydrophilic peptide to be added are integrated into an appropriate vector to express a modified epimorphin in a host. As a specific technique, there may be used, for example, the following simple technique.

cDNA (A) encoding a fragment containing the functional domain of epimorphin is first synthesized by polymerase chain reaction (PCR). On the other hand,

single-stranded DNAs (B and C), in which DNA encoding a hydrophilic peptide to be added to the N-terminus and/or C-terminus of the above fragment are joined to 10 to 20 bases on the N-terminal and C-terminal sides of the fragment containing the functional domain of epimorphin, respectively, are separately prepared by DNA synthesizer. A double-stranded DNA encoding the intended modified epimorphin is then obtained by PCR making use of the cDNA (A) as a template, and the DNAs (B) and (C) as primers. After the thus-obtained DNA is integrated into a proper site of an expression vector, gene transfer is performed into a proper host such as Escherichia coli or animal cells. The gene-transferred host is cultured under suitable conditions to induce the expression of the transgene, thereby preparing the modified epimorphin.

Since the modified epimorphin according to the present invention has a nature that it is easily dissolved in a physiological solution, its mass production and purification can be performed with ease. In addition, such a modified epimorphin highly keeps the epimorphin activity. The thus-obtained modified epimorphin is purified by an appropriate method, for example, affinity chromatography or the like, and then used as it is. When a plurality of modified epimorphin molecules is chemically crosslinked to each other to form a complex, however, higher activity may be exhibited in some cases. In this case, the crosslinking may be conducted with ease by an

irradiation treatment with ultraviolet (UV) rays or a chemical modification making use of a chemical substance such as glutaraldehyde or DSS (disuccinimidyl suberate). These crosslinking treatments may make the solubility of the resulting complex in physiological solutions lowered. For example, when the modified epimorphin is coated on a base material such as a plastic or metal to use, it is therefore necessary to give such considerations that the crosslinking treatment is performed after the coating of the modified epimorphin.

For example, the counting of the number of attached cells and the reactivity with the monoclonal antibody MC-1 may be used as indices to the evaluation of activity of the modified epimorphin. As examples of the method of counting the number of attached cells, may be mentioned the following methods. The modified epimorphin is suspended in a 8 M urea/Lysis buffer, and this suspension is coated on a suspension culture dish and then dried. Thereafter, the thus-coated dish is washed once with the 8 M urea/Lysis buffer and then 5 times with PBS. Cultured cells CH3/10T1/2 clone 8 (product of Dainippon Pharmaceutical Co., Ltd.) suspended in a D-MEM/F-12 medium (SIGMA D8900) added with 20 mg/ml of BSA (Bovine Serum Albumin) were then seeded on the coated dish. After left over for a predetermined period of time (for example, 4 hours), the dish is washed 3 times with PBS, and the cells are then lysed with 0.5 N NaOH, followed by recovery of

the resultant solution to measure the amount of DNA by a spectrophotometer, thereby counting the number of cells attached to the dish. Alternatively, an untreated polystyrene surface is coated with the modified epimorphin (10 μ/cm^2), and thoroughly washed with PBS. Thereafter, MDCKII cells (model of epithelial cells) suspended in a serum-free medium (Dulbecco MEM) containing 20 mg/ml of BSA are seeded on the surface. After left over for a predetermined period of time (for example, 8 hours), the number of cells attached to the surface is determined. These methods can also be applied to epimorphin and fragments of epimorphin.

As an index to the evaluation of solubility of the modified epimorphin, there may be used the amount of a fraction soluble in a physiological solution such as PBS. More specifically, the solubility may be evaluated by an amount of a soluble fraction when 1 mg of a protein is dissolved in 1 ml of PBS at 37°C. Therefore, the fact that 85% of the protein is soluble means that 0.85 mg of the protein is dissolved when 1 ml of PBS is added to 1 mg of the protein to mix them.

<Modified epimorphin composed of a polypeptide having a structure that at least part of amino acids have been deleted from the terminal side of at least one of the coiled coil domains (1) and (3)>

The central fragment (2) of epimorphin is a functional domain of epimorphin. Fragments containing the

functional domain (2) are expected to apply to various uses making good use of the epimorphin activity thereof. However, the polypeptides containing the functional domain (2) may be insoluble or hardly soluble in physiological solutions, or low in activity. When the C-terminal hydrophobic domain is deleted from the whole-length epimorphin, a polypeptide soluble in the physiological solutions can be obtained. However, such a polypeptide is low in activity.

As a result of a detailed analysis as to the activity and solubility of the fragments of epimorphin, it has been found that the fragment (123) is soluble, but extremely low in activity, and the fragment (23) is insoluble, but high in activity by contraries. From this, the present inventors have built up the following hypothesis as to the work of the fragment (1). Namely, the fragment (1) negatively works on activity due to the action to mask the functional domain, but works on the solubility to enhance it owing to the action to change the high-order structure of the fragment (23).

Thus, the present inventors have produced a 2M fragment obtained by deleting 28 amino acids (in the case of human epimorphin) or 29 amino acids (in the case of mouse epimorphin) from the N-terminal side of the fragment (123), a 3M fragment obtained by deleting 77 amino acids (in the case of human epimorphin) or 78 amino acids (in the case of mouse epimorphin) from the N-terminal side of

the fragment (123), and a fragment (23) as illustrated in FIG. 6 to analyze their properties. As a result, it has been revealed that the epimorphin fragments have a relationship that the activity increases, but on the other hand, the solubility decreases as the number of amino acids deleted from the N-terminal side is increased as illustrated in FIG. 6 (see the right of the drawing). It has hence been revealed that the activity and solubility of epimorphin polypeptides can be controlled by increasing or decreasing the number of amino acids to be deleted from the N-terminal side.

According to the present invention, as described above, there can be provided modified epimorphin polypeptides the activity and solubility of which can be controlled for the purpose of developing diagnosis and medical treatment for diseases caused by the morphogenetic abnormality of epithelial tissue, or novel remedies for wounds and the like.

More specifically, in the case where one attaches much importance to the activity, it is only necessary to produce a fragment with the number of amino acids deleted from the N-terminal side increased. In the case of human epimorphin, it is only necessary to produce a polypeptide having a structure that 78 to 103 amino acids, preferably, 91 to 103 amino acids have been deleted from the N-terminal side in order to obtain a modified epimorphin high in activity. In the case of mouse epimorphin, it is

only necessary to produce a polypeptide having a structure that 79 to 104 amino acids, preferably, 92 to 104 amino acids have been deleted from the N-terminal side in order to obtain a modified epimorphin high in activity. For example, the fragment (23) may preferably be used.

In the case where one attaches much importance to the solubility, it is only necessary to produce a fragment with the number of amino acids deleted from the N-terminal side decreased. In the case of human epimorphin, it is only necessary to produce a polypeptide having a structure that 1 to 28 amino acids, preferably, 14 to 28 amino acids have been deleted from the N-terminal side in order to obtain a modified epimorphin high in solubility. In the case of mouse epimorphin, it is only necessary to produce a polypeptide having a structure that 1 to 29 amino acids, preferably, 14 to 29 amino acids have been deleted from the N-terminal side in order to obtain a modified epimorphin high in activity. For example, the fragment (2M) may preferably be used.

In the case where a fragment well balanced between the activity and the solubility is intended to use, it is only necessary to produce a fragment in which the number of amino acid deleted from the N-terminal side is intermediate between the above ranges. In the case of human epimorphin, it is only necessary to produce a polypeptide having a structure that 29 to 77 amino acids, preferably, 61 to 77 amino acids have been deleted from

the N-terminal side. In the case of mouse epimorphin, it is only necessary to produce a polypeptide having a structure that 30 to 78 amino acids, preferably, 62 to 78 amino acids have been deleted from the N-terminal side. For example, the fragment (3M) may preferably be used.

As described above, the modified epimorphin according to the present may be used properly with its structure changed according to the purpose and application intended. Therefore, the use of the modified epimorphin according to the present invention allows the development of diagnosis and medical treatment for diseases caused by the morphogenetic abnormality of epithelial tissue, or the development of novel remedies for wounds and the like to more effectively progress. Epimorphin is a protein composed of about 280 amino acids. The mouse epimorphin is useful in, for example, elucidating the attack mechanism of diseases caused by the morphogenetic abnormality of the epithelial tissue making use of model animals. The human epimorphin is useful in, for example, diagnosing and treating such diseases. These molecules exist in mesenchymal cells around the epithelial tissue and have a function of controlling the morphogenesis of the epithelial tissue, and the like.

As a method of deleting amino acids from the N-terminal side of epimorphin, there may be used various techniques such as biochemical techniques and gene engineering techniques. As an example of a method making

use of a biochemical technique, may be mentioned a process in which an epimorphin molecule is chemically or physically cleaved to obtain the above-described fragments. As an example of a method making use of a gene engineering technique, may be mentioned a process in which cDNA encoding each of the fragments of epimorphin is integrated into an appropriate vector to express the fragment in a host. As a specific technique, there may be used, for example, the following simple technique.

cDNA (A) encoding the whole-length epimorphin is first synthesized by polymerase chain reaction (PCR). On the other hand, single-stranded DNAs (B and C), in which 10 to 20 bases on the N-terminal and C-terminal sides of the epimorphin fragment to be prepared are joined to one another, respectively, are separately prepared by a DNA synthesizer. A double-stranded DNA encoding the intended modified epimorphin is then obtained by PCR making use of the cDNA (A) as a template, and the DNAs (B) and (C) as primers.

The thus-obtained double-stranded DNA is integrated into a vector having a structure capable of expressing the DNA, for example, pET3C (RIKEN DNA Bank RDB519) to prepare a recombinant vector. This recombinant vector is then introduced into a proper host, for example, BL21 (RIKEN DNA Bank RDB022) to obtain a transformant. After propagating this transformant in a large amount, a treatment for inducing expression, for example, the

addition of IPTG to a medium so as to give a final concentration of 1 mM, is given to obtain the intended modified epimorphin.

DNA sequences encoding the fragments (2M), (3M), (23) and (123) of the human epimorphin are shown in the SEQUENCE TABLE. SEQ ID NOs. 7, 8, 9 and 10 are DNA sequences of the fragments (2M), (3M), (23) and (123) of the human epimorphin, respectively.

In the SEQUENCE TABLE, are shown DNA sequences encoding the fragments (2M), (3M), (23) and (123) of the mouse epimorphin. SEQ ID NOs. 11, 12, 13 and 14 are DNA sequences of the fragments (2M), (3M), (23) and (123) of the mouse epimorphin, respectively.

Amino acid sequences of the fragments (2M), (3M) and (23) of the human epimorphin are shown in the SEQUENCE TABLE. SEQ ID NOs. 15, 16 and 17 are amino acid sequences of the fragments (2M), (3M) and (23) of the human epimorphin, respectively.

Amino acid sequences of the fragments (2M), (3M) and (23) of the mouse epimorphin are shown in the SEQUENCE TABLE. SEQ ID NOs. 18, 19 and 20 are amino acid sequences of the fragments (2M), (3M) and (23) of the mouse epimorphin, respectively.

The modified epimorphin according to the present invention keeps the activity inherent in epimorphin at a high level, and is hence used directly in various applications including medical care, for example,

treatments of various tissues for burns or scalds, or after surgery, and artificial organs. Besides, it may also be used at a low concentration as a component for cosmetics, hair growth stimulants and the like as it is.

The modified epimorphin according to the present invention may be a variant obtained by making partial substitution, deletion and/or insertion of amino acids in the amino acid sequence of the modified epimorphin so far as it substantially keeps the epimorphin activity. The partial substitution, deletion and insertion of amino acids may be made either singly or in any combination thereof. The site of the amino acid sequence (the variable site), at which the partial substitution, deletion or insertion of amino acids is made, is generally in the amino acid sequence of a polypeptide containing the functional domain (2) of epimorphin. This variable site may be in the amino acid sequence of the functional domain (2) of epimorphin. Such a variant itself may be easily produced by the method known per se in the art. Namely, the technique itself, in which partial substitution, deletion or insertion is made in the amino acid sequence of a protein to obtain a variant of the protein, is generally known. For example, recombinant PCR ("PCR Protocols" 155-160, Harcourt Brace Javanovich Japan Inc. 1991) or producing method of recombinant gene with PCR ("Experimental Medicine" Vol. 8, No. 9, 63-67, Yodosha Co., Ltd. 1990). The variant modified epimorphin

according to the present invention may preferably substantially keep the functions inherent in the modified epimorphin, such as good cellular adhesiveness.

ADVANTAGES OF THE INVENTION

According to the present invention, the modified epimorphin which highly keeps the activity of epimorphin and are easy to prepare and purify is provided by adding a hydrophilic peptide to a polypeptide containing the functional domain (2) of epimorphin. Besides, according to the present invention, the modified epimorphin excellent in both activity and solubility is provided by deleting at least part of amino acids from the terminal side of at least one of the coiled coil domains (1) and (3). More specifically, the modified epimorphin which is soluble and far excellent in activity, or the modified epimorphin which is active and far excellent in solubility is provided. According to the present invention, there are further provided variants obtained by making partial substitution, deletion and/or insertion of amino acids in the amino acid sequence of the modified epimorphin. The modified epimorphin and variants thereof according to the present invention are soluble in physiological solutions and can hence be mass-produced and is easy to purify. The modified epimorphin and variants thereof of this invention keep the activity inherent in epiporphin at a high level, and is hence used directly in various applications

including medical care, for example, treatments of various tissues for burns or scalds, or after surgery, and artificial organs. Besides, they are useful as a component for cosmetics, hair growth stimulants and the like.

The proper use of the modified epimorphin and variants thereof according to the intended application in the research and medical fields allows the development of diagnosis and medical treatment for diseases caused by the morphogenetic abnormality of epithelial tissue, or the development of novel remedies for wounds and the like to more effectively progress.

EMBODIMENTS OF THE INVENTION

The present invention will hereinafter be described in more detail by the following examples.

Referential Example 1:

The analysis by a computer revealed that the constitutive amino acids of epimorphin are divided into four structurally-characteristic domains as illustrated in FIG. 1. Incidentally, the coiled coil domains (1) and (3) can each be further divided into four subfragments.

Thus, with respect to mouse epimorphin (polypeptide composed of 289 amino acids in the whole length) represented by SEQ ID NO. 4 of the SEQUENCE TABLE, peptide fragments corresponding to the respective domains were first designed as illustrated in FIG. 1 (fragments

indicated by 1, 2, 3, 12, 13, 23, 123 and 123C at lower rows in FIG. 1).

Respective cDNAs encoding the peptide fragments were separately prepared by PCR making use of the whole-length cDNA of the mouse epimorphin as a template, and separately inserted into the NdeI-BamH1 site of an expression vector PET3C [Gene, Vol. 56, 125-135 (1987)]. The thus-obtained vector/fragment cDNAs were separately introduced into Escherichia coli to induce them with IPTG (1 mM, 2 hours), thereby producing the respective epimorphin fragments in the Escherichia coli. Total proteins in the Escherichia coli were analyzed by SDS-PAGE. As a result, it was found that all the epimorphin fragments were produced in substantially equal amounts (see FIG. 2). These fragments were then electrically transferred to a nitrocellulose membrane to determine the functional site of epimorphin with a monoclonal antibody MC-1 [Cell, Vol. 69, 471-481 (1992)] which binds to the functional site. The domain situated at the center of the epimorphin molecule was found to correspond to the functional site (see FIG. 3).

Even in the fragments containing the functional domain, those containing a sequence on the N-terminal side or a hydrophobic domain composed of 23 to 24 amino acids adjacent to the C-terminus at the same time were found to be low in the reactivity to the antibody (namely, the functional site was masked). Therefore, the function was not smoothly exhibited (compare the fragments 12 and 123

with the fragment 2 and 123C in FIG. 3, respectively). The fragment indicated by 23 in FIG. 3 was extracted from the Escherichia coli to purify it. As a result, it was revealed that when amino acids up to 99th amino acid from the C-terminus are deleted from such a fragment, the solubility of epimorphin in a physiological solution (PBS) is significantly increased (the insolubility of at least 95% turned to the solubility of 40%). Namely, the fragment 23 is hardly soluble compared with the fragment 2. However, its solubility is increased by deleting the fragment 3.

On the basis of the results thus obtained, it was found that it is only necessary to contain an amino acid sequence ranging from the 100th amino acid to the 190th amino acid from the N-terminus in order to exhibit the function of epimorphin, that its function is gradually improved by successively deleting 30 to 99 amino acids from the N-terminal side, and that its solubility is gradually improved by successively deleting 25 to 99 amino acids from the C-terminal side.

Example 1:

A cDNA encoding a fragment (amino acid sequence ranging from 100th to 190th amino acids from the N-terminus) containing the functional domain of mouse epimorphin was prepared by PCR in the same manner as in Referential Example 1 and inserted into the NdeI or BamHl site of a vector PET3C [Gene, Vol. 56, 125-135 (1987)].

The thus-obtained vector/fragment cDNA was then introduced into Escherichia coli to produce polypeptides in which a peptide ASMTGGQQMGR was added as a hydrophilic peptide to the C-terminus or N-terminus of the above fragment.

The thus-obtained polypeptides were purified. As a result, all the polypeptides had a solubility of about 50% (namely, the solubility was improved by 10%). Even in the cases where peptides YPYDVPDYA, EQKLISEEDLN, EYKEEEEK, YTDIEMNRLGK, RIQRGPGRAFVTIGK, QPELAPEDPED and GAPVPYDPLEPR were separately added in accordance with the same procedure as described above, the solubility was recognized to be improved by about 5-15%. The polypeptides thus obtained were all able to be detected with good reproducibility by commercially-available specific antibodies.

On the other hand, a cDNA produced by selecting, as one of primers, a product obtained by connecting a single-stranded DNA encoding the sequence (ASMTGGQQMGR) composed of 11 amino acids to a positive-chain DNA encoding an amino acid sequence ranging from the 100th to 115th amino acids of epimorphin was inserted into the NdeI site of the above vector to conduct the same procedure as described above, thereby producing polypeptides in which the eleven amino acids were added to both C-terminus and N-terminus of the epimorphin functional domain-containing fragment. The thus-obtained polypeptides had a solubility of at least 85% in PBS.

MDCKII cells (model of epithelial cells) were suspended in a medium (Dulbecco MEM added with 10% serum) and then incubated for 8 hours in the presence of an equiamount of the soluble fraction of the epimorphin fragment. The thus-incubated cells were washed and then electrophoresed to detect the epimorphin fragments bound to the cells using an anti-ASMTGGQQMGR antibody (anti-T7-tag monoclonal antibody, product of Nobagen Co.). As a result, it was revealed that the epimorphin functional domain-containing fragment (modified epimorphin fragment) to both C-terminus and N-terminus of which the hydrophilic peptides composed of the eleven amino acids were added particularly firmly bound to the cells and then incorporated therein.

Example 2:

A single-stranded DNA (99 bases) encoding the 11 amino acid sequence (ASMTGGQQMGR) repeatedly 3 times was produced by a DNA synthesizer and then converted to a double-stranded DNA with DNA polymerase. This DNA was inserted repeatedly several times into PET3C vectors in which DNAs respectively encoding the epimorphin functional domain-containing fragments obtained in Example 1, to the C-terminus or N-terminus of which the amino acids had been added, had been separately inserted, thereby producing epimorphin functional domain-containing fragments, to both C-terminus and N-terminus of which amino acids of various lengths (11, 33, 66, 99, 44, 76 and 110) were added, in

Escherichia coli.

The function of the fragments thus obtained was evaluated with the MDCKII cells in the same manner as in Example 1. As a result, it was found that good cellular adhesiveness is recognized in the cases where the amino acids are added up to the number of 99 to both N- and C-termini, but the cellular adhesiveness tends to lower when the length of the amino acids added becomes longer than 99.

Example 3:

Polypeptides in which 3 to 15 consecutive His residues were added to the N-terminus of a fragment composed of the functional domain (amino acid sequence ranging from the 105th to 188th amino acids from the N-terminus) of mouse epimorphin were first produced in Escherichia coli in the same manner as in Example 1. The Escherichia coli strain thus obtained was lysed in 8 M urea to attempt one-step purification through a Ni-agarose column. As a result, it was possible to purify fragments without containing any other contaminant when a hydrophilic peptide containing at least 5 consecutive His residues was added.

Example 4:

Polypeptides in which 6 consecutive His residues were added to the N-termini of epimorphin fragments of various lengths containing the functional domain of mouse epimorphin were first produced in the same manner as in

Example 1. The respective fragments are shown in FIG. 4. In FIG. 4, the fragments are as follows:

123: a fragment ranging from the N-terminus to right before the C-terminal hydrophobic domain;

2M: a fragment ranging from the 30th amino acid from the N-terminus to right before the C-terminal hydrophobic domain;

3M: a fragment ranging from the 79th amino acid from the N-terminus to right before the C-terminal hydrophobic domain;

23: a fragment ranging from the 105th amino acid from the N-terminus to right before the C-terminal hydrophobic domain;

2: a fragment ranging from the 105th amino acid to the 188th amino acid from the N-terminus; and

13C: a fragment obtained by deleting the fragment 2 from the whole-length epimorphin.

These fragments were then purified through a Ni-agarose column and separately coated on culture dishes, and angioendothelial cells were then seeded on the dishes. As a result, the angioendothelial cells rapidly adhered to all the peptides containing the functional domain (the fragment 2). However, no cell adhesion was observed on the fragment obtained by deleting only the functional domain from the whole-length epimorphin.

The amounts of factors secreted by the cells adhered were determined. As a result, it was found that the

fragment obtained by adding the His residues to the fragment 123 is highest in activity.

Example 5:

An internal ear of a rabbit was first wounded in a size 6 mm across and 1 mm deep by a punch. This wound was treated with 1-10 μg of the modified epimorphin fragment (peptide containing the fragment 123) obtained in Example 4 and then taped. After 1 week, the tape was taken out of the wounded site to prepare slices from this wounded site, thereby determining reepithelialization rate, granulation rate and the number of neoplastic vessels. As a result, this modified epimorphin fragment was found to be useful in healing wounds. The use of the modified epimorphin fragment (peptide containing the fragment 3M) obtained in Example 4 gave the same results as described above. Therefore, it is understood that the modified epimorphin fragments containing the functional domain are useful in healing wounds.

Example 6:

The modified epimorphin fragment produced in Example 1, in which the hydrophilic peptide composed of the eleven amino acids (ASMTGGQQMGR) had been added to both termini thereof, was coated on two positions of an untreated polystyrene surface in density of 10 $\mu g/cm^2$. One of the coated portions was crosslinked with DSS (disuccinimidyl suberate). After the thus-coated surface was thoroughly washed with PBS, MDCKII cells suspended in a serum-free

medium (Dulbecco MEM) containing 20 mg/ml of BSA were seeded on the surface. After 2 hours and 8 hours, the number of cells attached to the fragments was determined. As a result, it was revealed that MDCKII rapidly and strongly bind to the crosslinked portion (see Table 1). Incidentally, the coating fragments on the polystyrene surface were dissolved in a sample buffer containing SDS without seeding the cells to collect them, thereby analyzing them by electrophoresis. As a result, it was confirmed that only the fragment subjected to the DSS treatment was polymerized.

Table 1

	Modified epimorphin obtained by adding the hydrophilic peptide composed of 11 amino acids to both termini of the epimorphin functional domain-containing fragment	Modified epimorphin obtained by adding the hydrophilic peptide composed of 11 amino acids to both termini of the epimorphin functional domain-containing fragment and crosslinking with DSS
Number of cells attached after 2 hours	10%	70%
Number of cells attached after 8 hours	85%	90%

(Note) The number of attached cells (%) is a value calculated out on the basis of the whole number (100%) of cells seeded.

Example 7:

Production of modified epimorphin

(1) Cell:

A mesenchymal cell strain expressing epimorphin, for example, CH3/10T1/2 clone 8 (Code No. 08-226, product of Dainippon Pharmaceutical Co., Ltd.) was purchased to culture it in accordance with the description thereof.

(2) Preparation of RNA:

A TRIzol Reagent (Cat. No. 15596-026) produced by Lifetec Oriental K.K. was used in the preparation of RNA. The RNA was prepared in accordance with a protocol attached to the product.

After the preparation, the RNA was treated with DNase I (Cat. No. 8068SA, amplification grade) produced by Lifetec Oriental K.K. in accordance with a protocol attached to the product.

(3) Preparation of epimorphin cDNA by RT-PCR:

On the basis of the RNA thus prepared, a reverse transcription was performed with an RNA PCR kit (Cat. No. R012) available from Takara Shuzo Co., Ltd. in accordance with a protocol attached to the product.

In order to amplify only cDNA of epimorphin, both upstream primer and downstream primer (5'ATGCGGGACCGGCTG3' and 5'TCATTTGCCAACCGA3') specific for epimorphin were then used to perform PCR in accordance with a protocol attached to the product, thereby obtain epimorphin cDNA. The preparation of the upstream and downstream primers specific for epimorphin was entrusted to Bex K.K.

(4) Preparation of fragments:

With respect to cDNAs encoding the fragments (2M), (3M), (23) and (123) of human epimorphin, base sequences of primers specific for the respective fragments were determined from SEQ ID NO. 7 (human 2M), SEQ ID NO. 8 (human 3M), SEQ ID NO. 9 (human 23), SEQ ID NO. 10 (human 123), and primers with a restriction enzyme NdeI site (5'CATATG3') and a restriction enzyme NheI site (5'GCTAGC3') tagged on the 5' side of the upstream primer and the 5' side of the downstream primer, respectively, were purchased from Bex K.K.

Similarly, with respect to cDNAs encoding the fragments (2M), (3M), (23) and (123) of mouse epimorphin, base sequences of primers specific for the respective fragments were determined from SEQ ID NO. 11 (mouse 2M), SEQ ID NO. 12 (mouse 3M), SEQ ID NO. 13 (mouse 23), SEQ ID NO. 14 (mouse 123), and primers with a restriction enzyme NdeI site (5'CATATG3') and a restriction enzyme NheI site (5'GCTAGC3') tagged on the 5' side of the upstream primer and the 5' side of the downstream primer, respectively, were purchased from Bex K.K.

The respective fragments were obtained by PCR making use of respective pairs of the primers. The PCR was performed with a Takara Taq (Cat. No. R001A) available from Takara Shuzo Co., Ltd. in accordance with a protocol attached to the product.

(5) Subcloning:

Each of the double-stranded DNAs thus obtained was integrated into an expression vector, for example, a vector derived from pET3C (RIKEN DNA Bank RDB519) by deleting a domain lying between two EcoRV sites thereof, in accordance with the process described in "Laboratory-Manual Gene Engineering", 111-114 (1988), published by Maruzen Co., Ltd., thereby producing a recombinant vector.

This recombinant vector was then introduced into a host, BL21 (RIKEN DNA Bank RDB022), in accordance with the Hanahan method described in "Laboratory-Manual Gene Engineering", 108-109, thereby obtaining transformants.

(6) Screening:

Colonies grown on an LB plate (1% Bacto-tryptone, 0.5% Bacto-yeast extract, 1% NaCl, 1.5% Bacto-agar) containing 50 μg/ml of ampicillin were selected to conduct the primary screening of the transformants.

In order to finally identify the transformants having the recombinant vector, the recombinant vector contained in the transformant was used as a template to perform PCR using the upstream and downstream primers specific for the modified epimorphin intended to ascertain whether the epimorphin cDNAs were present or not (at this point of time, 9 clones out of 10 clones contained the cDNAs).

After the transformants thus obtained were propagated in large amounts by shaking culture at 37°C on a liquid LB medium (1% Bacto-tryptone, 0.5% Bacto-yeast

extract, 1% NaCl) containing 50 μg/ml of ampicillin, a substance, IPTG, for inducing expression was added to the medium so as to give a final concentration of 1 mM. Thereafter, the shaking culture was continued for 2 hours at 37°C, thereby producing various human and mouse modified epimorphin polypeptides in Escherichia coli.

Total proteins in the Escherichia coli strain were analyzed by SDS-PAGE. As a result, it was found that all the modified epimorphin fragments were produced in substantially equal amounts.

Example 8:

Investigation as to solubility of the respective modified epimorphin polypeptides

Each of the transformants prepared in Example 7, which separately expressed the human and mouse modified epimorphin polypeptides, was suspended in a Lysis buffer [50 mM Tris-HCl (pH: 8.0), 1 mM EDTA, 100 mM NaCl] to wash it. The thus-washed transformant was subjected to centrifugation to precipitate the bacteria. After the thus-precipitated bacteria were suspended in a Lysis buffer, lysozyme (SIGMA L-6876) was added to the suspension to give a concentration of 1 mg/ml. The mixture was frozen and thawed repeatedly three times to lyse the Escherichia coli strain, followed by its ultrasonic treatment.

Thereafter, the supernatant was removed by centrifugation, and the resultant precipitate was washed 4

times with a 2 M urea/Lysis buffer. The thus-washed precipitate was suspended again in a 8 M urea/Lysis buffer, followed by centrifugation to obtain a supernatant fraction.

The thus-obtained supernatant fraction was dialyzed against an excess amount of a PBS buffer and further centrifuged, thereby separating it into a supernatant fraction and a precipitate fraction. The thus-obtained fractions were analyzed by SDS-PAGE to determine the solubility of each modified epimorphin by the proportions of the modified epimorphin existing in the respective fractions.

As a result, as illustrated in FIG. 6, there was shown a tendency for the modified epimorphin polypeptide to become more insoluble as the number of amino acids deleted from the N-terminal side thereof was increased.

Example 9:

Investigation as to activity of the respective modified epimorphin polypeptides

As an index to the activity evaluation of the respective modified epimorphin polypeptides, their binding ability to cultured cells was investigated.

The modified epimorphin polypeptides (suspensions in 8 M urea/Lysis buffer) prepared in Example 8 were separately coated on suspension culture dishes and then dried. Thereafter, the thus-coated dishes were washed once with 8 M urea/Lysis buffer and then 5 times with PBS.

Cultured cells CH3/10T1/2 clone 8 (product of Dainippon Pharmaceutical Co., Ltd.) suspended in a D-MEM/F-12 medium (SIGMA D8900) added with 20 mg/ml of BSA (SIGMA-A-7030) were then seeded on the coated dishes.

After left over for 1 hour and one day, the dishes were washed 3 times with PBS, and the cells were then lysed with 0.5 N NaOH. The resultant solutions were then recovered and further centrifuged. Thereafter, the amount of DNA in each of the solutions was measured by a spectrophotometer, thereby counting the number of cells attached to the dish to use this value as an index to the activity evaluation of the respective modified epimorphin polypeptides. As illustrated in FIG. 6, the results showed a tendency for the degree of activity to become opposite to the solubility.

Example 10:

Preparation of variant modified epimorphin

(A): cDNA encoding the fragment (2) of mouse epimorphin.

(B): Single-stranded DNA obtained by binding a base sequence capable of recognizing a restriction enzyme NdeI to the 5'-terminus of single-stranded DNA composed of 10 to 20 bases from the 5'-terminal side of a sense strand of (A).

(C): Single-stranded DNA obtained by binding a base sequence capable of recognizing a restriction enzyme NheI to the 5'-terminus of single-stranded DNA composed of 10

to 20 bases from the 5'-terminal side of an anti-sense strand of (A).

(1) (B) and (C) were produced by a DNA synthesizer.

(2) (A), and (B) and (C) were used as a template and primers, respectively, to obtain double-stranded DNA by a PCR process. At this time, the PCR was performed under conditions described in "Technique-a journal of methods in cell and molecular biology", Vol. 1, No. 1, 11-15 (August, 1989). By this process, DNAs encoding appropriate variant epimorphin fragments (2), in which substitution, variation occurred in part of the base sequence of (A), were obtained.

(3) The DNAs thus obtained were inserted into the NdeI/NheI site of pET3C, which was obtained by a domain lying between two EcoRV sites thereof, thereby producing a recombinant vector. This recombinant vector was then introduced into a host, Escherichia coli strain BL21 in accordance with the Hanahan process.

(4) This Escherichia coli strain was seeded on an LB plate (1% Bacto-tryptone, 0.5% Bacto-yeast extract, 1% NaCl, 1.5% Bacto-agar) containing 50 μg/ml of ampicillin to culture it overnight at 37°C.

(5) In order to identify colonies grown as transformants having the recombinant vector, an usual PCR process was performed to amplify the DNAs encoding the variant modified epimorphin fragments intended. Thereafter, the DNAs were subjected to agarose gel

electrophoresis, thereby confirming its bands.

(6) After the transformants thus obtained were propagated in large amounts by shaking culture at 37°C on liquid LB medium containing 50 μg/ml of ampicillin, substance, IPTG, for inducing expression was added to the medium so as to give a final concentration of 1 mM. Thereafter, the shaking culture was continued for 2 hours, thereby producing various variant modified epimorphin fragments in Escherichia coli.

(7) The Escherichia coli strain of (6) was suspended in Lysis buffer to wash it. The thus-washed strain was subjected to centrifugation to precipitate the bacteria and remove the supernatant. After the thus-precipitated bacteria were recovered and suspended in Lysis buffer, lysozyme was added to the suspension to give a concentration of 1 mg/ml. The mixture was frozen and thawed repeatedly three times to lyse the Escherichia coli strain, followed by its ultrasonic treatment. Thereafter, the supernatant was removed by centrifugation, and the resultant precipitate was washed 4 times with 2 M urea/Lysis buffer. The thus-washed precipitate was suspended again in 8 M urea/Lysis buffer, followed by centrifugation to obtain a supernatant fraction.

Example 11:

Determination of amino acid sequence of variant modified epimorphin

Plasmid DNAs in the Escherichia coli of the colonies

in (5) of Example 10 were prepared by the alkali process ["Laboratory-Manual Gene Engineering" 51-53 (1988), published by Maruzen Co., Ltd.] to analyze the base sequences of the DNAs encoding the variant modified epimorphin fragments by a DNA sequenser. Amino acid sequences encoded by these base sequences were determined to take them as amino acid sequences of their corresponding variant modified epimorphin fragments obtained in Example 10.

Example 12:

Evaluation of variant modified epimorphin in cell culture

(1) The variant modified epimorphin fragments (suspensions in 8 M urea/Lysis buffer) prepared in Example 10 were coated on suspension culture dishes to give a coating weight of 10 $\mu g/cm^2$, and then dried. Thereafter, the thus-coated dishes were washed once with 8 M urea/Lysis buffer and then 5 times with PBS. Cultured cells CH3/10T1/2 clone 8 suspended in a D-MEM/F-12 medium (SIGMA D8900) added with 20 mg/ml of BSA were then seeded on the coated dishes.

(2) After left over for 1 hour, the dishes were washed 3 times with PBS, and the cells were then recovered with 0.5 N NaOH. The value of OD at 260 nm, by which the amount of DNA contained therein was reflected, was determined by a spectrophotometer. The absorbance of an invariant modified epimorphin fragment was 0.33 ± 0.015. Among the variant modified epimorphin fragments obtained,

six fragments had an absorbance within about this range. This revealed that variants, in which part of their amino acid sequences have been varied, are permissible in the modified epimorphin.

The varied sites of the variant modified epimorphin fragments, which was produced and evaluated in Examples 10-12, are as shown in Table 2.

Table 2

Variant	Varied site (*1)	Original amino acid	Varied amino acid
a	149th 175th	Ile Ser	Val Pro
b	175th	Ser	Thr
c	115th 127th 130th 131th 139th 154th 166th 177th	Ile Phe Val Met Ile Glu Glu Phe	Val Leu Ala Thr Val Val Asp Leu
d	134th 171th 178th 179th 180th	Tyr Ser Ile Ser Asp	Phe Gly Thr Pro Gly
e	133th 145th 155th 162th 177th	Glu Ser Ile Asp Phe	Val Gly Asn Gly Ser
f	122th 115th 131th 155th	Asp Ile Met Ile	Gly Leu Val Phe

(*1) The number counted from the N-terminus of the whole-length mouse epimorphin.

SEQUENCE TABLE

SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 288 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
Met Arg Asp Arg Leu Pro Asp Leu Thr Ala Cys Arg Lys Asn Asp Asp
 1               5                  10                  15
Gly Asp Thr Val Val Val Val Glu Lys Asp His Phe Met Asp Asp Phe
            20                  25                  30
Phe His Gln Val Glu Glu Ile Arg Asn Ser Ile Asp Lys Ile Thr Gln
        35                  40                  45
Tyr Val Glu Glu Val Lys Lys Asn His Ser Ile Ile Leu Ser Ala Pro
    50                  55                  60
Asn Pro Glu Gly Lys Ile Lys Glu Glu Leu Glu Asp Leu Asn Lys Glu
65                  70                  75                  80
Ile Lys Lys Thr Ala Asn Lys Ile Arg Ala Lys Leu Lys Ala Ile Glu
                85                  90                  95
Gln Ser Phe Asp Gln Asp Glu Ser Gly Asn Arg Thr Ser Val Asp Leu
            100                 105                 110
Arg Ile Arg Arg Thr Gln His Ser Val Leu Ser Arg Lys Phe Val Glu
        115                 120                 125
Ala Met Ala Glu Tyr Asn Glu Ala Gln Thr Leu Phe Arg Glu Arg Ser
    130                 135                 140
Lys Gly Arg Ile Gln Arg Gln Leu Glu Ile Thr Gly Arg Thr Thr Thr
145                 150                 155                 160
```

SEQUENCE TABLE

SEQ ID NO : 1 :

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 288 amino acids

(B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : peptide

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 1 :

```
Met Arg Asp Arg Leu Pro Asp Leu Thr Ala Cys Arg Lys Asn Asp Asp
 1               5                  10                  15
Gly Asp Thr Val Val Val Val Glu Lys Asp His Phe Met Asp Asp Phe
            20                  25                  30
Phe His Gln Val Glu Glu Ile Arg Asn Ser Ile Asp Lys Ile Thr Gln
        35                  40                  45
Tyr Val Glu Glu Val Lys Lys Asn His Ser Ile Ile Leu Ser Ala Pro
    50                  55                  60
Asn Pro Glu Gly Lys Ile Lys Glu Glu Leu Glu Asp Leu Asn Lys Glu
65                  70                  75                  80
Ile Lys Lys Thr Ala Asn Lys Ile Arg Ala Lys Leu Lys Ala Ile Glu
                85                  90                  95
Gln Ser Phe Asp Gln Asp Glu Ser Gly Asn Arg Thr Ser Val Asp Leu
            100                 105                 110
Arg Ile Arg Arg Thr Gln His Ser Val Leu Ser Arg Lys Phe Val Glu
        115                 120                 125
Ala Met Ala Glu Tyr Asn Glu Ala Gln Thr Leu Phe Arg Glu Arg Ser
    130                 135                 140
Lys Gly Arg Ile Gln Arg Gln Leu Glu Ile Thr Gly Arg Thr Thr Thr
145                 150                 155                 160
```

Asp Asp Glu Leu Glu Glu Met Leu Glu Ser Gly Lys Pro Ser Ile Phe
165 170 175

Thr Ser Asp Ile Ile Ser Asp Ser Gln Ile Thr Arg Gln Ala Leu Asn
180 185 190

Glu Ile Glu Ser Arg His Lys Asp Ile Met Lys Leu Glu Thr Ser Ile
195 200 205

Arg Glu Leu His Glu Met Phe Met Asp Met Ala Met Phe Val Glu Thr
210 215 220

Gln Gly Glu Met Ile Asn Asn Ile Glu Arg Asn Val Met Asn Ala Thr
225 230 235 240

Asp Tyr Val Glu His Ala Lys Glu Glu Thr Lys Lys Ala Ile Lys Thr
245 250 255

Gln Ser Lys Ala Arg Arg Lys Lys Trp Ile Ile Ile Ala Val Ser Val
260 265 270

Val Leu Val Val Ile Ile Val Leu Ile Ile Gly Leu Ser Val Gly Lys
275 280 285

SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 287 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Arg Asp Arg Leu Pro Asp Leu Thr Ala Cys Arg Lys Asn Asp Asp
1 5 10 15

Gly Asp Thr Val Val Val Val Glu Lys Asp His Phe Met Asp Asp Phe
20 25 30
Phe His Gln Val Glu Glu Ile Arg Asn Ser Ile Asp Lys Ile Thr Gln
35 40 45
Tyr Val Glu Glu Val Lys Lys Asn His Ser Ile Ile Leu Ser Ala Pro
50 55 60
Asn Pro Glu Gly Lys Ile Lys Glu Glu Leu Glu Asp Leu Asn Lys Glu
65 70 75 80
Ile Lys Lys Thr Ala Asn Lys Ile Arg Ala Lys Leu Lys Ala Ile Glu
85 90 95
Gln Ser Phe Asp Gln Asp Glu Ser Gly Asn Arg Thr Ser Val Asp Leu
100 105 110
Arg Ile Arg Arg Thr Gln His Ser Val Leu Ser Arg Lys Phe Val Glu
115 120 125
Ala Met Ala Glu Tyr Asn Glu Ala Gln Thr Leu Phe Arg Glu Arg Ser
130 135 140
Lys Gly Arg Ile Gln Arg Gln Leu Glu Ile Thr Gly Arg Thr Thr Thr
145 150 155 160
Asp Asp Glu Leu Glu Glu Met Leu Glu Ser Gly Lys Pro Ser Ile Phe
165 170 175
Thr Ser Asp Ile Ile Ser Asp Ser Gln Ile Thr Arg Gln Ala Leu Asn
180 185 190
Glu Ile Glu Ser Arg His Lys Asp Ile Met Lys Leu Glu Thr Ser Ile
195 200 205
Arg Glu Leu His Glu Met Phe Met Asp Met Ala Met Phe Val Glu Thr
210 215 220
Gln Gly Glu Met Ile Asn Asn Ile Glu Arg Asn Val Met Asn Ala Thr
225 230 235 240

Gly Asp Thr Val Val Val Val Glu Lys Asp His Phe Met Asp Asp Phe
20 25 30
Phe His Gln Val Glu Glu Ile Arg Asn Ser Ile Asp Lys Ile Thr Gln
35 40 45
Tyr Val Glu Glu Val Lys Lys Asn His Ser Ile Ile Leu Ser Ala Pro
50 55 60
Asn Pro Glu Gly Lys Ile Lys Glu Glu Leu Glu Asp Leu Asn Lys Glu
65 70 75 80
Ile Lys Lys Thr Ala Asn Lys Ile Arg Ala Lys Leu Lys Ala Ile Glu
85 90 95
Gln Ser Phe Asp Gln Asp Glu Ser Gly Asn Arg Thr Ser Val Asp Leu
100 105 110
Arg Ile Arg Arg Thr Gln His Ser Val Leu Ser Arg Lys Phe Val Glu
115 120 125
Ala Met Ala Glu Tyr Asn Glu Ala Gln Thr Leu Phe Arg Glu Arg Ser
130 135 140
Lys Gly Arg Ile Gln Arg Gln Leu Glu Ile Thr Gly Arg Thr Thr Thr
145 150 155 160
Asp Asp Glu Leu Glu Glu Met Leu Glu Ser Gly Lys Pro Ser Ile Phe
165 170 175
Thr Ser Asp Ile Ile Ser Asp Ser Gln Ile Thr Arg Gln Ala Leu Asn
180 185 190
Glu Ile Glu Ser Arg His Lys Asp Ile Met Lys Leu Glu Thr Ser Ile
195 200 205
Arg Glu Leu His Glu Met Phe Met Asp Met Ala Met Phe Val Glu Thr
210 215 220
Gln Gly Glu Met Ile Asn Asn Ile Glu Arg Asn Val Met Asn Ala Thr
225 230 235 240

Asp Tyr Val Glu His Ala Lys Glu Glu Thr Lys Lys Ala Ile Lys Thr
245 250 255

Gln Ser Lys Ala Arg Arg Lys Leu Met Phe Ile Ile Ile Cys Val Ile
260 265 270

Val Leu Leu Val Ile Leu Gly Ile Ile Leu Ala Thr Thr Leu Ser
275 280 285

SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 277 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Met Arg Asp Arg Leu Pro Asp Leu Thr Ala Cys Arg Lys Asn Asp Asp
1 5 10 15

Gly Asp Thr Val Val Val Val Glu Lys Asp His Phe Met Asp Asp Phe
20 25 30

Phe His Gln Val Glu Glu Ile Arg Asn Ser Ile Asp Lys Ile Thr Gln
35 40 45

Tyr Val Glu Glu Val Lys Lys Asn His Ser Ile Ile Leu Ser Ala Pro
50 55 60

Asn Pro Glu Gly Lys Ile Lys Glu Glu Leu Glu Asp Leu Asn Lys Glu
65 70 75 80

Ile Lys Lys Thr Ala Asn Lys Ile Arg Ala Lys Leu Lys Ala Ile Glu
85 90 95

Gln Ser Phe Asp Gln Asp Glu Ser Gly Asn Arg Thr Ser Val Asp Leu
100 105 110

Arg Ile Arg Arg Thr Gln His Ser Val Leu Ser Arg Lys Phe Val Glu
115 120 125
Ala Met Ala Glu Tyr Asn Glu Ala Gln Thr Leu Phe Arg Glu Arg Ser
130 135 140
Lys Gly Arg Ile Gln Arg Gln Leu Glu Ile Thr Gly Arg Thr Thr Thr
145 150 155 160
Asp Asp Glu Leu Glu Glu Met Leu Glu Ser Gly Lys Pro Ser Ile Phe
165 170 175
Thr Ser Asp Ile Ile Ser Asp Ser Gln Ile Thr Arg Gln Ala Leu Asn
180 185 190
Glu Ile Glu Ser Arg His Lys Asp Ile Met Lys Leu Glu Thr Ser Ile
195 200 205
Arg Glu Leu His Glu Met Phe Met Asp Met Ala Met Phe Val Glu Thr
210 215 220
Gln Gly Glu Met Ile Asn Asn Ile Glu Arg Asn Val Met Asn Ala Thr
225 230 235 240
Asp Tyr Val Glu His Ala Lys Glu Glu Thr Lys Lys Ala Ile Lys Tyr
245 250 255
Gln Ser Lys Ala Arg Arg Gln Gln His Cys His Ser Asn His Ile Pro
260 265 270
Arg Ala Ile Tyr Pro
275

SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 289 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE : peptide

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 4 :

Met Arg Asp Arg Leu Pro Asp Leu Thr Ala Cys Arg Thr Asn Asp Asp
1 5 10 15

Gly Asp Thr Ala Val Val Ile Val Glu Lys Asp His Phe Met Asp Gly
20 25 30

Phe Phe His Gln Val Glu Glu Ile Arg Ser Ser Ile Ala Arg Ile Ala
35 40 45

Gln His Val Glu Asp Val Lys Lys Asn His Ser Ile Ile Leu Ser Ala
50 55 60

Pro Asn Pro Glu Gly Lys Ile Lys Glu Glu Leu Glu Asp Leu Asn Lys
65 70 75 80

Glu Ile Lys Lys Thr Ala Asn Arg Ile Arg Gly Lys Leu Lys Ser Ile
85 90 95

Glu Gln Ser Cys Asp Gln Asp Glu Asn Gly Asn Arg Thr Ser Val Asp
100 105 110

Leu Arg Ile Arg Arg Thr Gln His Ser Val Leu Ser Arg Lys Phe Val
115 120 125

Asp Val Met Thr Glu Tyr Asn Glu Ala Gln Ile Leu Phe Arg Glu Arg
130 135 140

Ser Lys Gly Arg Ile Gln Arg Gln Leu Glu Ile Thr Gly Arg Thr Thr
145 150 155 160

Thr Asp Asp Glu Leu Glu Glu Met Leu Glu Ser Gly Lys Pro Ser Ile
165 170 175

Phe Ile Ser Asp Ile Ile Ser Asp Ser Gln Ile Thr Arg Gln Ala Leu
180 185 190

Asn Glu Ile Glu Ser Arg His Lys Asp Ile Met Lys Leu Glu Thr Ser
195 200 205

Ile Arg Glu Leu His Glu Met Phe Met Asp Met Ala Met Phe Val Glu
210 215 220
Thr Gln Gly Glu Met Val Asn Asn Ile Glu Arg Asn Val Val Asn Ser
225 230 235 240
Val Asp Tyr Val Glu His Ala Lys Glu Glu Thr Lys Lys Ala Ile Lys
245 250 255
Tyr Gln Ser Lys Ala Arg Arg Lys Lys Trp Ile Ile Ala Ala Val Ala
260 265 270
Val Ala Val Ile Ala Val Leu Ala Leu Ile Ile Gly Leu Ser Val Gly
275 280 285
Lys

SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 288 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Met Arg Asp Arg Leu Pro Asp Leu Thr Ala Cys Arg Thr Asn Asp Asp
1 5 10 15
Gly Asp Thr Ala Val Val Ile Val Glu Lys Asp His Phe Met Asp Gly
20 25 30
Phe Phe His Gln Val Glu Glu Ile Arg Ser Ser Ile Ala Arg Ile Ala
35 40 45
Gln His Val Glu Asp Val Lys Lys Asn His Ser Ile Ile Leu Ser Ala
50 55 60

Pro Asn Pro Glu Gly Lys Ile Lys Glu Glu Leu Glu Asp Leu Asn Lys
65 70 75 80
Glu Ile Lys Lys Thr Ala Asn Arg Ile Arg Gly Lys Leu Lys Ser Ile
85 90 95
Glu Gln Ser Cys Asp Gln Asp Glu Asn Gly Asn Arg Thr Ser Val Asp
100 105 110
Leu Arg Ile Arg Arg Thr Gln His Ser Val Leu Ser Arg Lys Phe Val
115 120 125
Asp Val Met Thr Glu Tyr Asn Glu Ala Gln Ile Leu Phe Arg Glu Arg
130 135 140
Ser Lys Gly Arg Ile Gln Arg Gln Leu Glu Ile Thr Gly Arg Thr Thr
145 150 155 160
Thr Asp Asp Glu Leu Glu Glu Met Leu Glu Ser Gly Lys Pro Ser Ile
165 170 175
Phe Ile Ser Asp Ile Ile Ser Asp Ser Gln Ile Thr Arg Gln Ala Leu
180 185 190
Asn Glu Ile Glu Ser Arg His Lys Asp Ile Met Lys Leu Glu Thr Ser
195 200 205
Ile Arg Glu Leu His Glu Met Phe Met Asp Met Ala Met Phe Val Glu
210 215 220
Thr Gln Gly Glu Met Val Asn Asn Ile Glu Arg Asn Val Val Asn Ser
225 230 235 240
Val Asp Tyr Val Glu His Ala Lys Glu Glu Thr Lys Lys Ala Ile Lys
245 250 255
Tyr Gln Ser Lys Ala Arg Arg Lys Val Met Phe Val Leu Ile Cys Val
260 265 270
Val Thr Leu Leu Val Ile Leu Gly Ile Ile Leu Ala Thr Ala Leu Ser
275 280 285

SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 279 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
Met Arg Asp Arg Leu Pro Asp Leu Thr Ala Cys Arg Thr Asn Asp Asp
 1               5                  10                  15
Gly Asp Thr Ala Val Val Ile Val Glu Lys Asp His Phe Met Asp Gly
            20                  25                  30
Phe Phe His Gln Val Glu Glu Ile Arg Ser Ser Ile Ala Arg Ile Ala
        35                  40                  45
Gln His Val Glu Asp Val Lys Lys Asn His Ser Ile Ile Leu Ser Ala
    50                  55                  60
Pro Asn Pro Glu Gly Lys Ile Lys Glu Glu Leu Glu Asp Leu Asn Lys
65                  70                  75                  80
Glu Ile Lys Lys Thr Ala Asn Arg Ile Arg Gly Lys Leu Lys Ser Ile
                85                  90                  95
Glu Gln Ser Cys Asp Gln Asp Glu Asn Gly Asn Arg Thr Ser Val Asp
            100                 105                 110
Leu Arg Ile Arg Arg Thr Gln His Ser Val Leu Ser Arg Lys Phe Val
        115                 120                 125
Asp Val Met Thr Glu Tyr Asn Glu Ala Gln Ile Leu Phe Arg Glu Arg
    130                 135                 140
Ser Lys Gly Arg Ile Gln Arg Gln Leu Glu Ile Thr Gly Arg Thr Thr
145                 150                 155                 160
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Thr Asp Asp Glu Leu Glu Glu Met Leu Glu Ser Gly Lys Pro Ser Ile
165 170 175
Phe Ile Ser Asp Ile Ile Ser Asp Ser Gln Ile Thr Arg Gln Ala Leu
180 185 190
Asn Glu Ile Glu Ser Arg His Lys Asp Ile Met Lys Leu Glu Thr Ser
195 200 205
Ile Arg Glu Leu His Glu Met Phe Met Asp Met Ala Met Phe Val Glu
210 215 220
Thr Gln Gly Glu Met Val Asn Asn Ile Glu Arg Asn Val Val Asn Ser
225 230 235 240
Val Asp Tyr Val Glu His Ala Lys Glu Glu Thr Lys Lys Ala Ile Lys
245 250 255
Tyr Gln Ser Lys Ala Arg Arg Gln Gln His Cys His Ser Asn Arg Thr
260 265 270
Pro Arg Ala Leu Cys Pro Arg
275

SEQ ID NO : 7 :

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 237 base pairs

(B) TYPE : nucleic acid

(C) STRANDEDNESS : double

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE :

(A) ORGANISM : Homo sapiens

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 7 :

ATG GAT GAT TTC TTC CAT CAG GTG GAG GAG AAT AGA AAC AGT ATT GAT 16

AAA ATA ACT CAA TAT GTT GAA GAA GTA AAG AAA AAC CAC AGC ATC ATT 32

CTT TCT GCA CCA AAC CCG GAA GGA AAA ATA AAA GAA GAG CTT GAA GAT 48

CTG AAC AAA GAA ATC AAG AAA ACT GCG AAT AAA ATT CGA GCC AAG TTA 64

AAG GCT ATT GAA CAA AGT TTT GAT CAG GAT GAG AGT GGG AAC CGG ACT 80

TCA GTG GAT CTT CGG ATA CGA AGA ACC CAG CAT TCG GTG CTG TCT CGG 96

AAG TTT GTG GAA GCC ATG GCG GAG TAC AAT GAG GCA CAG ACT CTG TTT 112

CGG GAG CGG AGC AAA GGC CGC ATC CAG CGC CAG CTG GAG ATA ACT GGG 128

AGA ACC ACC ACA GAC GAC GAG CTA GAA GAG ATG CTG GAG AGC GGG AAG 144

CCA TCC ATC TTC ACT TCC GAC ATT ATA TCA GAT TCA CAA ATT ACT AGA 160

CAA GCT CTC AAT GAA ATC GAG TCA CGT CAC AAG GAC ATC ATG AAG CTG 176

GAG ACC AGC ATC CGA GAG TTG CAT GAG ATG TTC ATG GAC ATG GCT ATG 192

TTT GTG GAG ACT CAG GGT GAA ATG ATC AAC AAC ATA GAA AGA AAT GTT 208

ATG AAT GCC ACA GAC TAT GTA GAA CAC GCT AAA GAA GAA ACA AAA AAA 224

GCT ATC AAA TAT CAG AGC AAG GCA AGA AGG AAA AAG TGA 237

SEQ ID NO : 8 :

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 188 base pairs

(B) TYPE : nucleic acid

(C) STRANDEDNESS : double

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE :

(A) ORGANISM : Homo sapiens

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 8 :

AAC AAA GAA ATC AAG AAA ACT GCG AAT AAA ATT CGA GCC AAG TTA AAG 16

GCT ATT GAA CAA AGT TTT GAT CAG GAT GAG AGT GGG AAC CGG ACT TCA 32

GTG GAT CTT CGG ATA CGA AGA ACC CAG CAT TCG GTG CTG TCT CGG AAG 48

TTT GTG GAA GCC ATG GCG GAG TAC AAT GAG GCA CAG ACT CTG TTT CGG 64

GAG CGG AGC AAA GGC CGC ATC CAG CGC CAG CTG GAG ATA ACT GGG AGA 80

ACC ACC ACA GAC GAC GAG CTA GAA GAG ATG CTG GAG AGC GGG AAG CCA 96

TCC ATC TTC ACT TCC GAC ATT ATA TCA GAT TCA CAA ATT ACT AGA CAA 112

GCT CTC AAT GAA ATC GAG TCA CGT CAC AAG GAC ATC ATG AAG CTG GAG 128

ACC AGC ATC CGA GAG TTG CAT GAG ATG TTC ATG GAC ATG GCT ATG TTT 144

GTG GAG ACT CAG GGT GAA ATG ATC AAC AAC ATA GAA AGA AAT GTT ATG 160

AAT GCC ACA GAC TAT GTA GAA CAC GCT AAA GAA GAA ACA AAA AAA GCT 176

ATC AAA TAT CAG AGC AAG GCA AGA AGG AAA AAG TGA 188

SEQ ID NO : 9 :

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 162 base pairs

(B) TYPE : nucleic acid

(C) STRANDEDNESS : double

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE :

(A) ORGANISM : Homo sapiens

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 9 :

AGT GGG AAC CGG ACT TCA GTG GAT CTT CGG ATA CGA AGA ACC CAG CAT 16

TCG GTG CTG TCT CGG AAG TTT GTG GAA GCC ATG GCG GAG TAC AAT GAG 32

GCA CAG ACT CTG TTT CGG GAG CGG AGC AAA GGC CGC ATC CAG CGC CAG 48

CTG GAG ATA ACT GGG AGA ACC ACC ACA GAC GAC GAG CTA GAA GAG ATG 64

CTG GAG AGC GGG AAG CCA TCC ATC TTC ACT TCC GAC ATT ATA TCA GAT 80

TCA CAA ATT ACT AGA CAA GCT CTC AAT GAA ATC GAG TCA CGT CAC AAG 96

GAC ATC ATG AAG CTG GAG ACC AGC ATC CGA GAG TTG CAT GAG ATG TTC 112

ATG GAC ATG GCT ATG TTT GTG GAG ACT CAG GGT GAA ATG ATC AAC AAC 128

ATA GAA AGA AAT GTT ATG AAT GCC ACA GAC TAT GTA GAA CAC GCT AAA 144
GAA GAA ACA AAA AAA GCT ATC AAA TAT CAG AGC AAG GCA AGA AGG AAA 160
AAG TGA 162

SEQ ID NO : 10 :

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 265 base pairs

(B) TYPE : nucleic acid

(C) STRANDEDNESS : double

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE :

(A) ORGANISM : Homo sapiens

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 10 :

ATG CGG GAC CGG CTG CCA GAC CTG ACG GCG TGT AGG AAG AAT GAT GAT 16
GGA GAC ACA GTT GTT GTG GTT GAG AAA GAT CAT TTC ATG GAT GAT TTC 32
TTC CAT CAG GTG GAG GAG ATT AGA AAC AGT ATT GAT AAA ATA ACT CAA 48
TAT GTT GAA GAA GTA AAG AAA AAC CAC AGC ATC ATT CTT TCT GCA CCA 64
AAC CCG GAA GGA AAA ATA AAA GAA GAG CTT GAA GAT CTG AAC AAA GAA 80
ATC AAG AAA ACT GCG AAT AAA ATT CGA GCC AAG TTA AAG GCT ATT GAA 96
CAA AGT TTT GAT CAG GAT GAG AGT GGG AAC CGG ACT TCA GTG GAT CTT 112
CGG ATA CGA AGA ACC CAG CAT TCG GTG CTG TCT CGG AAG TTT GTG GAA 128
GCC ATG GCG GAG TAC AAT GAG GCA CAG ACT CTG TTT CGG GAG CGG AGC 144
AAA GGC CGC ATC CAG CGC CAG CTG GAG ATA ACT GGG AGA ACC ACC ACA 160
GAC GAC GAG CTA GAA GAG ATG CTG GAG AGC GGG AAG CCA TCC ATC TTC 176
ACT TCC GAC ATT ATA TCA GAT TCA CAA ATT ACT AGA CAA GCT CTC AAT 192
GAA ATC GAG TCA CGT CAC AAG GAC ATC ATG AAG CTG GAG ACC AGC ATC 208
CGA GAG TTG CAT GAG ATG TTC ATG GAC ATG GCT ATG TTT GTG GAG ACT 224

CAG GGT GAA ATG ATC AAC AAC ATA GAA AGA AAT GTT ATG AAT GCC ACA 240
GAC TAT GTA GAA CAC GCT AAA GAA GAA ACA AAA AAA GCT ATC AAA TAT 256
CAG AGC AAG GCA AGA AGG AAA AAG TGA 265

SEQ ID NO : 11 :

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 237 base pairs

(B) TYPE : nucleic acid

(C) STRANDEDNESS : double

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE :

(A) ORGANISM : Mus

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 11 :

ATG GAC GGT TTC TTC CAT CAG GTA GAG GAG ATT CGA AGC AGC ATA GCC 16
AGG ATT GCT CAG CAT GTA GAA GAC GTG AAG AAG AAC CAC AGC ATC ATC 32
CTG TCT GCT CCA AAC CCA GAA GGA AAA ATA AAA GAA GAG CTG GAG GAC 48
CTG AAC AAA GAG ATC AAG AAA ACT GCT AAC AGG ATC CGG GGC AAG CTG 64
AAG TCT ATT GAG CAG AGC TGT GAT CAG GAC GAG AAT GGG AAC CGA ACT 80
TCA GTG GAT CTG CGG ATA CGA AGG ACC CAG CAC TCG GTG CTG TCA CGG 96
AAG TTT GTG GAC GTC ATG ACA GAA TAC AAT GAA GCG CAG ATC CTG TTC 112
CGG GAG CGA AGC AAA GGC CGC ATC CAG CGC CAG CTG GAG ATC ACT GGG 128
AGG ACC ACC ACT GAC GAC GAG CTG GAA GAG ATG CTG GAG AGC GGG AAG 144
CCG TCC ATC TTC ATC TCG GAT ATT ATA TCA GAT TCA CAA ATC ACT AGG 160
CAA GCT CTC AAT GAG ATC GAG TCC CGC CAC AAA GAC ATC ATG AAG CTG 176
GAG ACC AGC ATC CGA GAG CTG CAC GAG ATG TTC ATG GAT ATG GCC ATG 192
TTT GTC GAG ACT CAG GGT GAA ATG GTC AAC AAC ATC GAG AGA AAT GTG 208
GTG AAC TCT GTA GAT TAC GTG GAA CAT GCC AAG GAA GAG ACG AAG AAA 224

GCC ATC AAA TAC CAG AGC AAG GCC AGG CGG AAA AAG TGA 237

SEQ ID NO : 12 :

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 188 base pairs

(B) TYPE : nucleic acid

(C) STRANDEDNESS : double

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE :

(A) ORGANISM : Mus

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 12 :

AAC AAA GAG ATC AAG AAA ACT GCT AAC AGG ATC CGG GGC AAG CTG AAG 16

TCT ATT GAG CAG AGC TGT GAT CAG GAC GAG AAT GGG AAC CGA ACT TCA 32

GTG GAT CTG CGG ATA CGA AGG ACC CAG CAC TCG GTG CTG TCA CGG AAG 48

TTT GTG GAC GTC ATG ACA GAA TAC AAT GAA GCG CAG ATC CTG TTC CGG 64

GAG CGA AGC AAA GGC CGC ATC CAG CGC CAG CTG GAG ATC ACT GGG AGG 80

ACC ACC ACT GAC GAC GAG CTG GAA GAG ATG CTG GAG AGC GGG AAG CCG 96

TCC ATC TTC ATC TCG GAT ATT ATA TCA GAT TCA CAA ATC ACT AGG CAA 112

GCT CTC AAT GAG ATC GAG TCC CGC CAC AAA GAC ATC ATG AAG CTG GAG 128

ACC AGC ATC CGA GAG CTG CAC GAG ATG TTC ATG GAT ATG GCC ATG TTT 144

GTC GAG ACT CAG GGT GAA ATG GTC AAC AAC ATC GAG AGA AAT GTG GTG 160

AAC TCT GTA GAT TAC GTG GAA CAT GCC AAG GAA GAG ACG AAG AAA GCC 176

ATC AAA TAC CAG AGC AAG GCC AGG CGG AAA AAG TGA 188

SEQ ID NO : 13 :

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 162 base pairs

(B) TYPE : nucleic acid

(C) STRANDEDNESS : double

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE :

(A) ORGANISM : Mus

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 13 :

AAT GGG AAC CGA ACT TCA GTG GAT CTG CGG ATA CGA AGG ACC CAG CAC 16

TCG GTG CTG TCA CGG AAG TTT GTG GAC GTC ATG ACA GAA TAC AAT GAA 32

GCG CAG ATC CTG TTC CGG GAG CGA AGC AAA GGC CGC ATC CAG CGC CAG 48

CTG GAG ATC ACT GGG AGG ACC ACC ACT GAC GAC GAG CTG GAA GAG ATG 64

CTG GAG AGC GGG AAG CCG TCC ATC TTC ATC TCG GAT ATT ATA TCA GAT 80

TCA CAA ATC ACT AGG CAA GCT CTC AAT GAG ATC GAG TCC CGC CAC AAA 96

GAC ATC ATG AAG CTG GAG ACC AGC ATC CGA GAG CTG CAC GAG ATG TTC 112

ATG GAT ATG GCC ATG TTT GTC GAG ACT CAG GGT GAA ATG GTC AAC AAC 128

ATC GAG AGA AAT GTG GTG AAC TCT GTA GAT TAC GTG GAA CAT GCC AAG 144

GAA GAG ACG AAG AAA GCC ATC AAA TAC CAG AGC AAG GCC AGG CGG AAA 160

AAG TGA 162

SEQ ID NO : 14 :

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 266 base pairs

(B) TYPE : nucleic acid

(C) STRANDEDNESS : double

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE :

(A) ORGANISM : Mus

(B) TYPE : nucleic acid

(C) STRANDEDNESS : double

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE :

(A) ORGANISM : Mus

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 13 :

AAT GGG AAC CGA ACT TCA GTG GAT CTG CGG ATA CGA AGG ACC CAG CAC 16

TCG GTG CTG TCA CGG AAG TTT GTG GAC GTC ATG ACA GAA TAC AAT GAA 32

GCG CAG ATC CTG TTC CGG GAG CGA AGC AAA GGC CGC ATC CAG CGC CAG 48

CTG GAG ATC ACT GGG AGG ACC ACC ACT GAC GAC GAG CTG GAA GAG ATG 64

CTG GAG AGC GGG AAG CCG TCC ATC TTC ATC TCG GAT ATT ATA TCA GAT 80

TCA CAA ATC ACT AGG CAA GCT CTC AAT GAG ATC GAG TCC CGC CAC AAA 96

GAC ATC ATG AAG CTG GAG ACC AGC ATC CGA GAG CTG CAC GAG ATG TTC 112

ATG GAT ATG GCC ATG TTT GTC GAG ACT CAG GGT GAA ATG GTC AAC AAC 128

ATC GAG AGA AAT GTG GTG AAC TCT GTA GAT TAC GTG GAA CAT GCC AAG 144

GAA GAG ACG AAG AAA GCC ATC AAA TAC CAG AGC AAG GCC AGG CGG AAA 160

AAG TGA 162

SEQ ID NO : 14 :

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 266 base pairs

(B) TYPE : nucleic acid

(C) STRANDEDNESS : double

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE :

(A) ORGANISM : Mus

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 14 :

ATG CGG GAC CGG CTG CCC GAC CTC ACG GCG TGT AGG ACA AAC GAC GAT 16
GGA GAC ACT GCT GTC GTC ATT GTG GAG AAG GAT CAT TTC ATG GAC GGT 32
TTC TTC CAT CAG GTA GAG GAG ATT CGA AGC AGC ATA GCC AGG ATT GCT 48
CAG CAT GTA GAA GAC GTG AAG AAG AAC CAC AGC ATC ATC CTG TCT GCT 64
CCA AAC CCA GAA GGA AAA ATA AAA GAA GAG CTG GAG GAC CTG GAC AAA 80
GAG ATC AAG AAA ACT GCT AAC AGG ATC CGG GGC AAG CTG AAG TCT ATT 96
GAG CAG AGC TGT GAT CAG GAC GAG AAT GGG AAC CGA ACT TCA GTG GAT 112
CTG CGG ATA CGA AGG ACC CAG CAC TCG GTG CTG TCA CGG AAG TTT GTG 128
GAC GTC ATG ACA GAA TAC AAT GAA GCG CAG ATC CTG TTC CGG GAG CGA 144
AGC AAA GGC CGC ATC CAG CGC CAG CTG GAG ATC ACT GGG AGG ACC ACC 160
ACT GAC GAC GAG CTG GAA GAG ATG CTG GAG AGC GGG AAG CCG TCC ATC 176
TTC ATC TCG GAT ATT ATA TCA GAT TCA CAA ATC ACT AGG CAA GCT CTC 192
AAT GAG ATC GAG TCC CGC CAC AAA GAC ATC ATG AAG CTG GAG ACC AGC 208
ATC CGA GAG CTG CAC GAG ATG TTC ATG GAT ATG GCC ATG TTT GTC GAG 224
ACT CAG GGT GAA ATG GTC AAC AAC ATC GAG AGA AAT GTG GTG AAC TCT 240
GTA GAT TAC GTG GAA CAT GCC AAG GAA GAG ACG AAG AAA GCC ATC AAA 256
TAC CAG AGC AAG GCC AGG CGG AAA AAG TGA 266

SEQ ID NO : 15 :

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 236 amino acids

(B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : peptide

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 15 :

Met Asp Asp Phe Phe His Gln Val Glu Glu Ile Arg Asn Ser Ile Asp
1 5 10 15

Lys Ile Thr Gln Tyr Val Glu Glu Val Lys Lys Asn His Ser Ile Ile
20 25 30
Leu Ser Ala Pro Asn Pro Glu Gly Lys Ile Lys Glu Glu Leu Glu Asp
35 40 45
Leu Asn Lys Glu Ile Lys Lys Thr Ala Asn Lys Ile Arg Ala Lys Leu
50 55 60
Lys Ala Ile Glu Gln Ser Phe Asp Gln Asp Glu Ser Gly Asn Arg Thr
65 70 75 80
Ser Val Asp Leu Arg Ile Arg Arg Thr Gln His Ser Val Leu Ser Arg
85 90 95
Lys Phe Val Glu Ala Met Ala Glu Tyr Asn Glu Ala Gln Thr Leu Phe
100 105 110
Arg Glu Arg Ser Lys Gly Arg Ile Gln Arg Gln Leu Glu Ile Thr Gly
115 120 125
Arg Thr Thr Thr Asp Asp Glu Leu Glu Glu Met Leu Glu Ser Gly Lys
130 135 140
Pro Ser Ile Phe Thr Ser Asp Ile Ile Ser Asp Ser Gln Ile Thr Arg
145 150 155 160
Gln Ala Leu Asn Glu Ile Glu Ser Arg His Lys Asp Ile Met Lys Leu
165 170 175
Glu Thr Ser Ile Arg Glu Leu His Glu Met Phe Met Asp Met Ala Met
180 185 190
Phe Val Glu Thr Gln Gly Glu Met Ile Asn Asn Ile Glu Arg Asn Val
195 200 205
Met Asn Ala Thr Asp Tyr Val Glu His Ala Lys Glu Glu Thr Lys Lys
210 215 220
Ala Ile Lys Thr Gln Ser Lys Ala Arg Arg Lys Lys
225 230 235

SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 187 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Asn Lys Glu Ile Lys Lys Thr Ala Asn Lys Ile Arg Ala Lys Leu Lys
1 5 10 15

Ala Ile Glu Gln Ser Phe Asp Gln Asp Glu Ser Gly Asn Arg Thr Ser
20 25 30

Val Asp Leu Arg Ile Arg Arg Thr Gln His Ser Val Leu Ser Arg Lys
35 40 45

Phe Val Glu Ala Met Ala Glu Tyr Asn Glu Ala Gln Thr Leu Phe Arg
50 55 60

Glu Arg Ser Lys Gly Arg Ile Gln Arg Gln Leu Glu Ile Thr Gly Arg
65 70 75 80

Thr Thr Thr Asp Asp Glu Leu Glu Glu Met Leu Glu Ser Gly Lys Pro
85 90 95

Ser Ile Phe Thr Ser Asp Ile Ile Ser Asp Ser Gln Ile Thr Arg Gln
100 105 110

Ala Leu Asn Glu Ile Glu Ser Arg His Lys Asp Ile Met Lys Leu Glu
115 120 125

Thr Ser Ile Arg Glu Leu His Glu Met Phe Met Asp Met Ala Met Phe
130 135 140

Val Glu Thr Gln Gly Glu Met Ile Asn Asn Ile Glu Arg Asn Val Met
145 150 155 160

Asn Ala Thr Asp Tyr Val Glu His Ala Lys Glu Glu Thr Lys Lys Ala
165 170 175

Ile Lys Thr Gln Ser Lys Ala Arg Arg Lys Lys
180 185

SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 161 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Ser Gly Asn Arg Thr Ser Val Asp Leu Arg Ile Arg Arg Thr Gln His
1 5 10 15

Ser Val Leu Ser Arg Lys Phe Val Glu Ala Met Ala Glu Tyr Asn Glu
20 25 30

Ala Gln Thr Leu Phe Arg Glu Arg Ser Lys Gly Arg Ile Gln Arg Gln
35 40 45

Leu Glu Ile Thr Gly Arg Thr Thr Thr Asp Asp Glu Leu Glu Glu Met
50 55 60

Leu Glu Ser Gly Lys Pro Ser Ile Phe Thr Ser Asp Ile Ile Ser Asp
65 70 75 80

Ser Gln Ile Thr Arg Gln Ala Leu Asn Glu Ile Glu Ser Arg His Lys
85 90 95

Asp Ile Met Lys Leu Glu Thr Ser Ile Arg Glu Leu His Glu Met Phe
100 105 110

Met Asp Met Ala Met Phe Val Glu Thr Gln Gly Glu Met Ile Asn Asn
115 120 125

Ile Glu Arg Asn Val Met Asn Ala Thr Asp Tyr Val Glu His Ala Lys
130 135 140
Glu Glu Thr Lys Lys Ala Ile Lys Thr Gln Ser Lys Ala Arg Arg Lys
145 150 155 160
Lys

SEQ ID NO : 18 :

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 236 amino acids

(B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : peptide

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 18 :

Met Asp Gly Phe Phe His Gln Val Glu Glu Ile Arg Ser Ser Ile Ala
1 5 10 15
Arg Ile Ala Gln His Val Glu Asp Val Lys Lys Asn His Ser Ile Ile
20 25 30
Leu Ser Ala Pro Asn Pro Glu Gly Lys Ile Lys Glu Glu Leu Glu Asp
35 40 45
Leu Asn Lys Glu Ile Lys Lys Thr Ala Asn Arg Ile Arg Gly Lys Leu
50 55 60
Lys Ser Ile Glu Gln Ser Cys Asp Gln Asp Glu Asn Gly Asn Arg Thr
65 70 75 80
Ser Val Asp Leu Arg Ile Arg Arg Thr Gln His Ser Val Leu Ser Arg
85 90 95
Lys Phe Val Asp Val Met Thr Glu Tyr Asn Glu Ala Gln Ile Leu Phe
100 105 110

Ile Glu Arg Asn Val Met Asn Ala Thr Asp Tyr Val Glu His Ala Lys
130 135 140
Glu Glu Thr Lys Lys Ala Ile Lys Thr Gln Ser Lys Ala Arg Arg Lys
145 150 155 160
Lys

SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 236 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Met Asp Gly Phe Phe His Gln Val Glu Glu Ile Arg Ser Ser Ile Ala
1 5 10 15
Arg Ile Ala Gln His Val Glu Asp Val Lys Lys Asn His Ser Ile Ile
20 25 30
Leu Ser Ala Pro Asn Pro Glu Gly Lys Ile Lys Glu Glu Leu Glu Asp
35 40 45
Leu Asn Lys Glu Ile Lys Lys Thr Ala Asn Arg Ile Arg Gly Lys Leu
50 55 60
Lys Ser Ile Glu Gln Ser Cys Asp Gln Asp Glu Asn Gly Asn Arg Thr
65 70 75 80
Ser Val Asp Leu Arg Ile Arg Arg Thr Gln His Ser Val Leu Ser Arg
85 90 95
Lys Phe Val Asp Val Met Thr Glu Tyr Asn Glu Ala Gln Ile Leu Phe
100 105 110

Ile Glu Arg Asn Val Met Asn Ala Thr Asp Tyr Val Glu His Ala Lys
130 135 140
Glu Glu Thr Lys Lys Ala Ile Lys Thr Gln Ser Lys Ala Arg Arg Lys
145 150 155 160
Lys

SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 236 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Met Asp Gly Phe Phe His Gln Val Glu Glu Ile Arg Ser Ser Ile Ala
1 5 10 15
Arg Ile Ala Gln His Val Glu Asp Val Lys Lys Asn His Ser Ile Ile
20 25 30
Leu Ser Ala Pro Asn Pro Glu Gly Lys Ile Lys Glu Glu Leu Glu Asp
35 40 45
Leu Asn Lys Glu Ile Lys Lys Thr Ala Asn Arg Ile Arg Gly Lys Leu
50 55 60
Lys Ser Ile Glu Gln Ser Cys Asp Gln Asp Glu Asn Gly Asn Arg Thr
65 70 75 80
Ser Val Asp Leu Arg Ile Arg Arg Thr Gln His Ser Val Leu Ser Arg
85 90 95
Lys Phe Val Asp Val Met Thr Glu Tyr Asn Glu Ala Gln Ile Leu Phe
100 105 110

Arg Glu Arg Ser Lys Gly Arg Ile Gln Arg Gln Leu Glu Ile Thr Gly
115 120 125
Arg Thr Thr Thr Asp Asp Glu Leu Glu Glu Met Leu Glu Ser Gly Lys
130 135 140
Pro Ser Ile Phe Ile Ser Asp Ile Ile Ser Asp Ser Gln Ile Thr Arg
145 150 155 160
Gln Ala Leu Asn Glu Ile Glu Ser Arg His Lys Asp Ile Met Lys Leu
165 170 175
Glu Thr Ser Ile Arg Glu Leu His Glu Met Phe Met Asp Met Ala Met
180 185 190
Phe Val Glu Thr Gln Gly Glu Met Val Asn Asn Ile Glu Arg Asn Val
195 200 205
Val Asn Ser Val Asp Tyr Val Glu His Ala Lys Glu Glu Thr Lys Lys
210 215 220
Ala Ile Lys Tyr Gln Ser Lys Ala Arg Arg Lys Lys
225 230 235

SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 187 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Asn Lys Glu Ile Lys Lys Thr Ala Asn Arg Ile Arg Gly Lys Leu Lys
1 5 10 15
Ser Ile Glu Gln Ser Cys Asp Gln Asp Glu Asn Gly Asn Arg Thr Ser
20 25 30

Val Asp Leu Arg Ile Arg Arg Thr Gln His Ser Val Leu Ser Arg Lys
35 40 45
Phe Val Asp Val Met Thr Glu Tyr Asn Glu Ala Gln Ile Leu Phe Arg
50 55 60
Glu Arg Ser Lys Gly Arg Ile Gln Arg Gln Leu Glu Ile Thr Gly Arg
65 70 75 80
Thr Thr Thr Asp Asp Glu Leu Glu Glu Met Leu Glu Ser Gly Lys Pro
85 90 95
Ser Ile Phe Ile Ser Asp Ile Ile Ser Asp Ser Gln Ile Thr Arg Gln
100 105 110
Ala Leu Asn Glu Ile Glu Ser Arg His Lys Asp Ile Met Lys Leu Glu
115 120 125
Thr Ser Ile Arg Glu Leu His Glu Met Phe Met Asp Met Ala Met Phe
130 135 140
Val Glu Thr Gln Gly Glu Met Val Asn Asn Ile Glu Arg Asn Val Val
145 150 155 160
Asn Ser Val Asp Tyr Val Glu His Ala Lys Glu Glu Thr Lys Lys Ala
165 170 175
Ile Lys Tyr Gln Ser Lys Ala Arg Arg Lys Lys
180 185

SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 161 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Asn Gly Asn Arg Thr Ser Val Asp Leu Arg Ile Arg Arg Thr Gln His
1 5 10 15
Ser Val Leu Ser Arg Lys Phe Val Asp Val Met Thr Glu Tyr Asn Glu
20 25 30
Ala Gln Ile Leu Phe Arg Glu Arg Ser Lys Gly Arg Ile Gln Arg Gln
35 40 45
Leu Glu Ile Thr Gly Arg Thr Thr Thr Asp Asp Glu Leu Glu Glu Met
50 55 60
Leu Glu Ser Gly Lys Pro Ser Ile Phe Ile Ser Asp Ile Ile Ser Asp
65 75 75 80
Ser Gln Ile Thr Arg Gln Ala Leu Asn Glu Ile Glu Ser Arg His Lys
85 90 95
Asp Ile Met Lys Leu Glu Thr Ser Ile Arg Glu Leu His Glu Met Phe
100 105 110
Met Asp Met Ala Met Phe Val Glu Thr Gln Gly Glu Met Val Asn Asn
115 125 125
Ile Glu Arg Asn Val Val Asn Ser Val Asp Tyr Val Glu His Ala Lys
130 135 140
Glu Glu Thr Lys Lys Ala Ile Lys Tyr Gln Ser Lys Ala Arg Arg Lys
145 150 155 160
Lys

Claimed:

1. A modified epimorphin obtained by adding a hydrophilic peptide composed of 5 to 99 amino acids to at least one terminus of a polypeptide containing the functional domain of epimorphin.

2. The modified epimorphin according to Claim 1, wherein the functional domain of epimorphin is defined as a central fragment (2) obtained by deleting a coiled coil domain (1) on the N-terminal side, a coiled coil domain (3) on the C-terminal side and a hydrophobic domain adjacent to the C-terminus from the whole-length epimorphin.

3. The modified epimorphin according to Claim 1 or 2, wherein the polypeptide containing the functional domain of epimorphin is the whole-length epimorphin.

4. The modified epimorphin according to Claim 1 or 2, wherein the polypeptide containing the functional domain of epimorphin is a fragment obtained by deleting part of amino acids from the whole-length epimorphin from the side of at least one terminus thereof.

5. The modified epimorphin according to Claim 4, wherein the polypeptide containing the functional domain

of epimorphin is a fragment a obtained by deleting at least part of amino acids in the coiled coil domain (1) from the whole-length epimorphin from the N-terminal side thereof.

6. The modified epimorphin according to Claim 4, wherein the polypeptide containing the functional domain of epimorphin is a fragment b obtained by deleting the C-terminal hydrophobic domain from the whole-length epimorphin.

7. The modified epimorphin according to Claim 4, wherein the polypeptide containing the functional domain of epimorphin is a fragment c obtained by deleting the C-terminal hydrophobic domain from the whole-length epimorphin and moreover deleting at least part of amino acids in the coiled coil domain (1) thereof from the N-terminal side.

8. The modified epimorphin according to Claim 4, wherein the polypeptide containing the functional domain of epimorphin is a fragment d obtained by deleting the C-terminal hydrophobic domain from the whole-length epimorphin and moreover deleting at least part of amino acids in the coiled coil domain (3) thereof from the C-terminal side.

9. The modified epimorphin according to Claim 4, wherein the polypeptide containing the functional domain of epimorphin is a fragment e obtained by deleting the C-terminal hydrophobic domain from the whole-length epimorphin and moreover deleting at least part of amino acids in the coiled coil domain (1) thereof from the N-terminal side and at least part of amino acids in the coiled coil domain (3) thereof from the C-terminal side.

10. The modified epimorphin according to Claim 3, wherein the hydrophilic peptide is added to both termini of the whole-length epimorphin.

11. The modified epimorphin according to Claim 5, wherein the hydrophilic peptide is added to at least the N-terminus out of both termini of the fragment a.

12. The modified epimorphin according to Claim 6, wherein the hydrophilic peptide is added to at least the N-terminus out of both termini of the fragment b.

13. The modified epimorphin according to Claim 7, wherein the hydrophilic peptide is added to at least the N-terminus out of both termini of the fragment c.

14. The modified epimorphin according to Claim 8, wherein the hydrophilic peptide is added to at least the

C-terminus out of both termini of the fragment d.

15. The modified epimorphin according to any one of Claims 1 to 14, wherein the hydrophilic peptide contains bound hydrophilic amino acids in a proportion of at least 50% based on the number of amino acids.

16. The modified epimorphin according to any one of Claims 1 to 15, wherein the hydrophilic peptide contains at least one amino acid sequence selected from the group consisting of the following amino acid sequences (a) to (h):

(a) Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala;

(b) Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu-Asn;

(c) Glu-Tyr-Lys-Glu-Glu-Glu-Glu-Lys;

(d) Tyr-Thr-Asp-Ile-Glu-Met-Asn-Arg-Leu-Gly-Lys;

(e) Arg-Ile-Gln-Arg-Gly-Pro-Gly-Arg-Ala-Phe-Val-Thr-Ile-Gly-Lys;

(f) Ala-Ser-Met-Thr-Gly-Gly-Gln-Gln-Met-Gly-Arg;

(g) Gln-Pro-Glu-Leu-Ala-Pro-Glu-Asp-Pro-Glu-Asp; and

(h) Gly-Ala-Pro-Val-Pro-Tyr-Asp-Pro-Leu-Glu-Pro-Arg.

17. The modified epimorphin according to any one of Claims 1 to 15, wherein the hydrophilic peptide contains at least 5 consecutive histidine residues.

18. The modified epimorphin according to any one of

Claims 1 to 17, which has been subjected to intermolecular crosslinking to form a complex.

19. The modified epimorphin according to any one of Claims 1 to 18, wherein the epimorphin is human epimorphin.

20. The modified epimorphin according to Claim 19, wherein the human epimorphin is one selected from the group consisting of epimorphin represented by SEQ ID NO. 1, epimorphin isoform A represented by SEQ ID NO. 2 and epimorphin isoform B represented by SEQ ID NO. 3, all shown in SEQUENCE TABLE.

21. The modified epimorphin according to Claim 19 or 20, wherein the polypeptide containing the functional domain of epimorphin contains at least an amino acid sequence ranging from the 99th amino acid to the 189th amino acid from the N-terminus of the human epimorphin.

22. The modified epimorphin according to Claim 19 or 20, wherein the polypeptide containing the functional domain of epimorphin contains at least an amino acid sequence ranging from the 104th amino acid to the 187th amino acid from the N-terminus of the human epimorphin.

23. The modified epimorphin according to Claim 19 or

20, wherein the polypeptide containing the functional domain of epimorphin is a fragment obtained by deleting 1 to 28 amino acids in the coiled coil domain (1) from the whole-length human epimorphin from the N-terminal side and deleting the C-terminal hydrophobic domain therefrom.

24. The modified epimorphin according to Claim 19 or 20, wherein the polypeptide containing the functional domain of epimorphin is a fragment obtained by deleting 29 to 103 amino acids from the whole-length human epimorphin from the N-terminal side.

25. The modified epimorphin according to Claim 24, wherein the polypeptide containing the functional domain of epimorphin is a fragment obtained by deleting 29 to 77 amino acids in the coiled coil domain (1) from the whole-length human epimorphin from the N-terminal side and deleting the C-terminal hydrophobic domain therefrom.

26. The modified epimorphin according to Claim 24, wherein the polypeptide containing the functional domain of epimorphin is a fragment obtained by deleting 78 to 103 amino acids in the coiled coil domain (1) from the whole-length human epimorphin from the N-terminal side and deleting the C-terminal hydrophobic domain therefrom.

27. The modified epimorphin according to Claim 19 or

20, wherein the polypeptide containing the functional domain of epimorphin is a fragment obtained by deleting 30 to 98 amino acids in the coiled coil domain (1) from the whole-length human epimorphin from the N-terminal side and deleting the C-terminal hydrophobic domain therefrom.

28. The modified epimorphin according to any one of Claims 1 to 18, wherein the epimorphin is mouse epimorphin.

29. The modified epimorphin according to Claim 28, wherein the mouse epimorphin is one selected from the group consisting of epimorphin represented by SEQ ID NO. 4, epimorphin isoform A represented by SEQ ID NO. 5 and epimorphin isoform B represented by SEQ ID NO. 6, all shown in SEQUENCE TABLE.

30. The modified epimorphin according to Claim 28 or 29, wherein the polypeptide containing the functional domain of epimorphin contains at least an amino acid sequence ranging from the 100th amino acid to the 190th amino acid from the N-terminus of the mouse epimorphin.

31. The modified epimorphin according to Claim 28 or 29, wherein the polypeptide containing the functional domain of epimorphin contains at least an amino acid sequence ranging from the 105th amino acid to the 188th

amino acid from the N-terminus of the mouse epimorphin.

32. The modified epimorphin according to Claim 28 or 29, wherein the polypeptide containing the functional domain of epimorphin is a fragment obtained by deleting 1 to 29 amino acids in the coiled coil domain (1) from the whole-length mouse epimorphin from the N-terminal side and deleting the C-terminal hydrophobic domain therefrom.

33. The modified epimorphin according to Claim 28 or 29, wherein the polypeptide containing the functional domain of epimorphin is a fragment obtained by deleting 30 to 104 amino acids from the whole-length mouse epimorphin from the N-terminal side.

34. The modified epimorphin according to Claim 33, wherein the polypeptide containing the functional domain of epimorphin is a fragment obtained by deleting 30 to 78 amino acids in the coiled coil domain (1) from the whole-length mouse epimorphin from the N-terminal side and deleting the C-terminal hydrophobic domain therefrom.

35. The modified epimorphin according to Claim 33, wherein the polypeptide containing the functional domain of epimorphin is a fragment obtained by deleting 79 to 104 amino acids in the coiled coil domain (1) from the whole-length mouse epimorphin from the N-terminal side and

deleting the C-terminal hydrophobic domain therefrom.

36. The modified epimorphin according to Claim 28 or 29, wherein the polypeptide containing the functional domain of epimorphin is a fragment obtained by deleting 30 to 99 amino acids in the coiled coil domain (1) from the whole-length mouse epimorphin from the N-terminal side and deleting the C-terminal hydrophobic domain therefrom.

37. A modified epimorphin composed of a polypeptide having a structure that a hydrophobic domain adjacent to the C-terminus of the whole-length epimorphin consisting of a coiled coil domain (1) on the N-terminal side, a functional domain (2) at the center, a coiled coil domain (3) on the C-terminal side and the hydrophobic domain (4) adjacent to the C-terminus has been deleted from the whole-length epimorphin, and at least part of amino acids have been deleted from the terminal side of at least one of the coiled coil domains (1) and (3) as well.

38. The modified epimorphin according to Claim 37, which is composed of a polypeptide having a structure that at least part of amino acids of the coiled coil domain (1) have been deleted from the N-terminal side.

39. The modified epimorphin according to Claim 37 or 38, wherein the epimorphin is human epimorphin.

40. The modified epimorphin according to Claim 39, which is composed of a polypeptide having a structure that 1 to 28 amino acids have been deleted form the N-terminal side of the coiled coil domain (1).

41. The modified epimorphin according to Claim 39, which is composed of a polypeptide having a structure that 29 to 77 amino acids have been deleted form the N-terminal side of the coiled coil domain (1).

42. The modified epimorphin according to Claim 39, which is composed of a polypeptide having a structure that 78 to 103 amino acids have been deleted form the N-terminal side of the coiled coil domain (1).

43. The modified epimorphin according to Claim 39, which is a human modified epimorphin represented by any one of amino acid sequences of SEQ ID NO. 15 to 17 shown in SEQUENCE TABLE.

44. The modified epimorphin according to Claim 37 or 38, wherein the epimorphin is mouse epimorphin.

45. The modified epimorphin according to Claim 44, which is composed of a polypeptide having a structure that 1 to 29 amino acids have been deleted form the N-terminal side of the coiled coil domain (1).

46. The modified epimorphin according to Claim 44, which is composed of a polypeptide having a structure that 30 to 78 amino acids have been deleted form the N-terminal side of the coiled coil domain (1).

47. The modified epimorphin according to Claim 44, which is composed of a polypeptide having a structure that 79 to 104 amino acids have been deleted form the N-terminal side of the coiled coil domain (1).

48. The modified epimorphin according to Claim 44, which is a mouse modified epimorphin represented by any one of amino acid sequences of SEQ ID NO. 18 to 20 shown in SEQUENCE TABLE.

49. DNA encoding the modified epimorphin according to any one of Claims 1 to 48.

50. The DNA according to Claim 49, wherein the modified epimorphin is one according to any one of Claims 1 to 36.

51. The DNA according to Claim 49, wherein the modified epimorphin is one according to any one of Claims 37 to 48.

52. The DNA according to Claim 51, which is DNA

encoding a human modified epimorphin represented by any one of base sequences of SEQ ID NOs. 7 to 9 shown in SEQUENCE TABLE.

53. The DNA according to Claim 51, which is DNA encoding a mouse modified epimorphin represented by any one of base sequences of SEQ ID NOs. 11 to 13 shown in SEQUENCE TABLE.

54. A recombinant vector which contains DNA encoding the modified epimorphin according to any one of Claims 1 to 48 and is capable of expressing its corresponding polypeptide.

55. The recombinant vector according to Claim 54, wherein the DNA is DNA encoding the modified epimorphin according to any one of Claims 1 to 36.

56. The recombinant vector according to Claim 54, wherein the DNA is DNA encoding the modified epimorphin according to any one of Claims 37 to 48.

57. The recombinant vector according to Claim 56, which contains DNA encoding a human modified epimorphin represented by any one of base sequences of SEQ ID NOs. 7 to 9 shown in SEQUENCE TABLE, and is capable of expressing its corresponding polypeptide.

58. The recombinant vector according to Claim 56, which contains DNA encoding a mouse modified epimorphin represented by any one of base sequences of SEQ ID NOs. 11 to 13 shown in SEQUENCE TABLE, and is capable of expressing its corresponding polypeptide.

59. A transformant obtained by introducing a recombinant vector which contains DNA encoding the modified epimorphin according to any one of Claims 1 to 48 and is capable of expressing its corresponding polypeptide.

60. The transformant according to Claim 59, wherein the recombinant vector contains DNA encoding the modified epimorphin according to any one of Claims 1 to 36.

61. The transformant according to Claim 59, wherein the recombinant vector contains DNA encoding the modified epimorphin according to any one of Claims 37 to 48.

62. The transformant according to Claim 61, which is obtained by introducing a recombinant vector containing DNA encoding a human modified epimorphin represented by any one of base sequences of SEQ ID NOs. 7 to 9 shown in SEQUENCE TABLE, said vector being capable of expressing its corresponding polypeptide.

63. The transformant according to Claim 61, which is obtained by introducing a recombinant vector containing DNA encoding a mouse modified epimorphin represented by any one of base sequences of SEQ ID NOs. 11 to 13 shown in SEQUENCE TABLE, said vector being capable of expressing its corresponding polypeptide.

64. The transformant according to any one of Claims 59 to 63, wherein a host of the transformant is Escherichia coli.

65. A method of producing a modified epimorphin, comprising using a transformant obtained by introducing a recombinant vector which contains DNA encoding the modified epimorphin according to any one of Claims 1 to 48 and is capable of expressing its corresponding polypeptide.

66. The method according to Claim 65, wherein the transformant is obtained by introducing a recombinant vector which contains DNA encoding the modified epimorphin according to any one of Claims 1 to 36 and is capable of expressing its corresponding polypeptide.

67. The method according to Claim 65, wherein the transformant is obtained by introducing a recombinant vector which contains DNA encoding the modified epimorphin

according to any one of Claims 37 to 48 and is capable of expressing its corresponding polypeptide.

68. The method according to Claim 67 for producing a human modified epimorphin, which comprises using a transformant obtained by introducing a recombinant vector containing DNA encoding a human modified epimorphin represented by any one of base sequences of SEQ ID NOs. 7 to 9 shown in SEQUENCE TABLE, said vector being capable of expressing its corresponding polypeptide.

69. The method according to Claim 67 for producing a mouse modified epimorphin, which comprises using a transformant obtained by introducing a recombinant vector containing DNA encoding a human modified epimorphin represented by any one of base sequences of SEQ ID NOs. 11 to 13 shown in SEQUENCE TABLE, said vector being capable of expressing its corresponding polypeptide.

70. A variant modified epimorphin obtained by making partial substitution, deletion and/or insertion of amino acids in the amino acid sequence of the modified epimorphin according to any one of Claims 1 to 48, wherein the variant maintains the function of the original sequence.

71. The variant modified epimorphin according to

Claim 70, wherein the site of the amino acid sequence, at which the partial substitution, deletion or insertion of amino acids is made in the amino acid sequence of the modified epimorphin, is in the amino acid sequence of a polypeptide containing the functional domain of epimorphin.

72. The variant modified epimorphin according to Claim 70, wherein the site of the amino acid sequence, at which the partial substitution, deletion or insertion of amino acids is made in the amino acid sequence of the modified epimorphin, is in the amino acid sequence of the functional domain of epimorphin.

73. DNA encoding the variant modified epimorphin according to any one of Claims 70 to 72.

74. A recombinant vector which contains DNA encoding the variant modified epimorphin according to Claim 73, and is capable of expressing its corresponding polypeptide.

75. A transformant obtained by introducing the recombinant vector according to Claim 74.

76. A method of producing a variant modified epimorphin, comprising using the transformant according to Claim 75.

Fig. 1

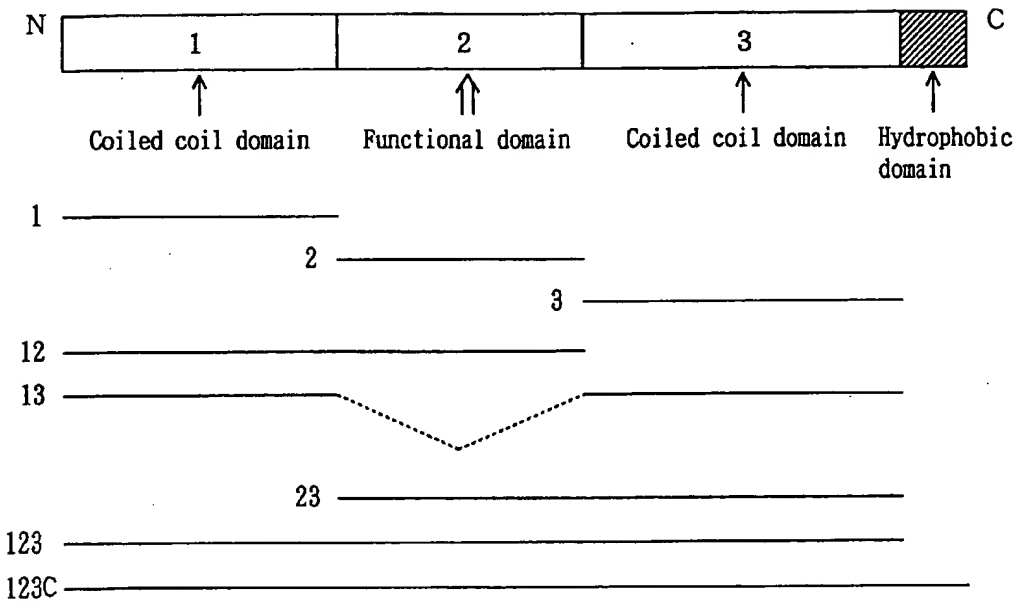
N
1
2
3
C
Coiled coil domain
Functional domain
Coiled coil domain
Hydrophobic domain
1
2
3
12
13
23
123
123C

Fig. 2

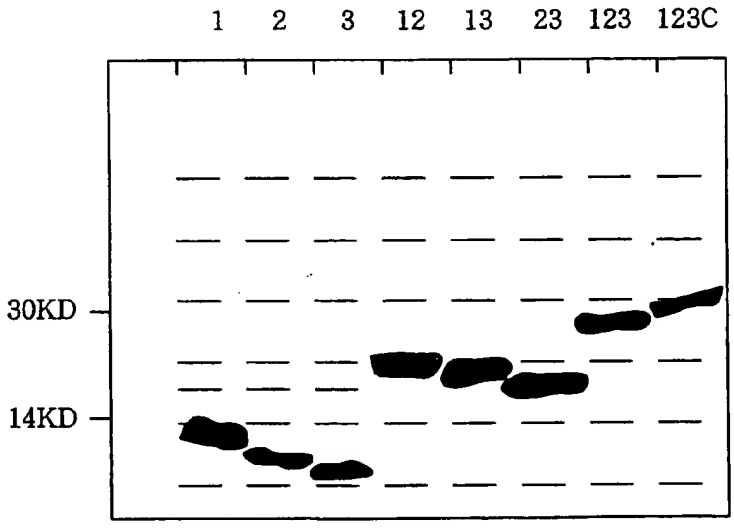
1
2
3
12
13
23
123
123C
30KD
14KD

Fig. 3

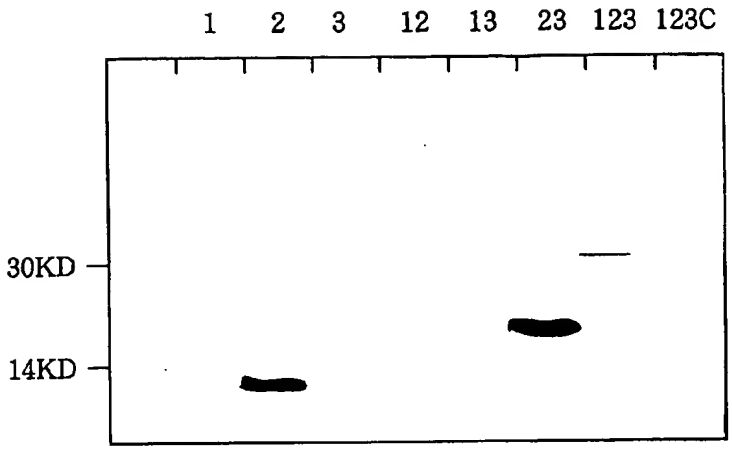

Fig. 4

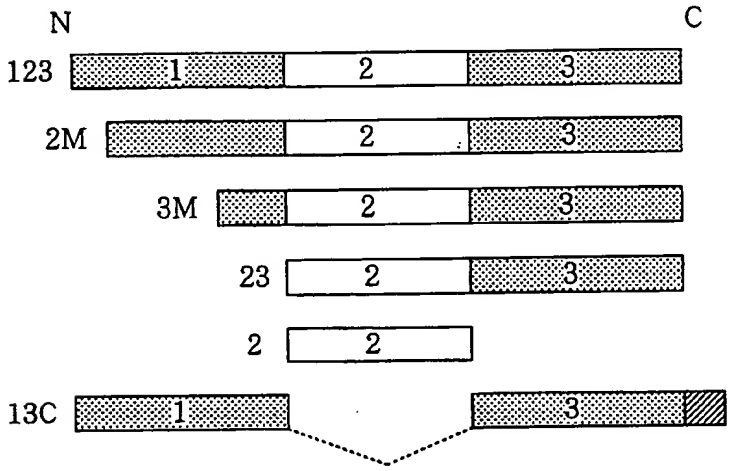

Fig. 5

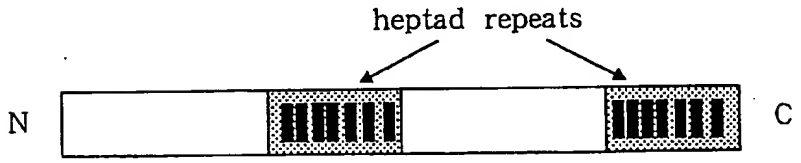

Fig. 6